European Union Reference Laboratory for Fish Diseases

National Veterinary Institute, Technical University of Denmark, Copenhagen

Technical Report 2015

from the European Union Reference Laboratory for Fish Diseases

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European Union Reference Laboratory Antimicrobial Resistance

National Veterinary Institute
Technical University of Denmark
Section for Virology
Copenhagen, Denmark

DTU

Content

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Annex 1: Reagents received at the EURL in 2015

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Introduction

The National Veterinary Institute, Technical University of Denmark (DTU-VET) is appointed as the European Union Reference Laboratory for Fish Diseases (EURL), in accordance with the Commission Implementing Regulation (EU) No 135/2013 of 18 February 2013, the notification of grant decision for an action regarding the EU Reference Laboratory for Fish Diseases – SI2.700369 and the corresponding grand decision (SG/ck/sante.ddg2.g.5(2015)) as regards the Union financial aid for the year 2015 to the EURL Fish Diseases

The duties of the EURL are described in Council Directive 2006/88/EC of 24 October 2006 (Annex VI). The duties mainly concern fish diseases listed as exotic diseases: epizootic haematopoietic necrosis (EHN); and fish diseases listed as non-exotic diseases: infectious salmon anaemia (ISA), viral haemorrhagic septicaemia (VHS), infectious haematopoietic necrosis (IHN) and koi herpes virus disease (KHVD). This report follows the format of the work programme adopted for the EURL for 2015, describing activities and sub-activities and the status of on-going projects. The list of functions and duties of the EURL follows this introduction.
The 19th Annual Meeting of the National Reference Laboratories for Fish Diseases was held in Copenhagen, Denmark, 27th-28th May at the premises of the Veterinary Institute. A total of 53 participants from 33 countries attended over the two days period. There were five sessions with a total of 25 presentations, 3 of which were given by invited speakers, and a working group session.

Again this year an inter-laboratory proficiency test was distributed to the NRLs mainly within the EU but there were also participants from countries outside EU. For the fifth year, the proficiency test consisted of two tests, PT1 and PT2. PT1 was designed to primarily assess the ability of participating laboratories to identify VHSV, IHNV and EHNV. PT2 was developed in order to test the proficiency of participating laboratories to identify ISA and KHV; in 2015 the identification of SAV was included as well on a voluntary base. The proficiency test is covering all 5 listed exotic and non-exotic diseases. 45 National Reference Laboratories (NRLs) participated in the proficiency test. A report was submitted primo March 2016. Most laboratories performed well.

An important focus of the EURL is to update the standard operating procedures of the non-exotic and exotic listed diseases. Diagnostic manuals for VHS, IHN, ISA, KHV and EHN are available at the EURL web page.
The final version of the Commission Decision 2015-1554 on sampling and diagnostic procedures for all non-exotic diseases listed in Council Directive 2006/88/EC have finally been adopted and the procedures for the fish diseases are available on the EURL webpage http://www.eurl-fish.eu/Diagnostic_Manuals.

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

During 2015, resources were again used to collate data on surveillance, health categorisation, and diagnostics in EU; to identify and characterise selected virus isolates; to type, store and update a library of listed virus isolates; to develop, update and maintain the database containing information on fish pathogens (www.fishpathogens.eu); to supply reference materials to NRLs; to provide training courses in laboratory diagnosis; to update the EURL webpage (www.eurl-fish.eu); and finally to attend international meetings and conferences.

This report was prepared and collated in a close collaboration between EURL Coordinator Niccoló Vendramin, molecular biologist Susie Sommer Mikkelsen, and undersigned with contributions from the other academic staff in the Group for Fish Diseases.

Frederiksberg, 30 March 2016

Niels Jørgen Olesen
Professor, DVM
Head of EURL for Fish Diseases

The functions and duties for the European Union Reference Laboratory for Fish Diseases

According to Council Directive 2006/88/EC of 24 October 2006 - Annex VI.
Period: 1 January 2015 – 31 December 2015

The functions and duties for the European Union Reference Laboratory for Fish Diseases (EURL)

The European Union reference laboratories shall:

(a) coordinate, in consultation with the Commission, the methods employed in the Member States for diagnosing the disease concerned, specifically by:

(i) typing, storing and, where appropriate, supplying strains of the pathogen of the relevant disease to facilitate the diagnostic service in the Community,

(ii) supplying standard sera and other reference reagents to the national reference laboratories in order to standardize the tests and reagents used in each Member State, where serological tests are required, L 328/48 EN Official Journal of the European Union 24.11.2006

(iii) organising periodic comparative tests (ring tests) of diagnostic procedures at Community level with the national reference laboratories designated by the Member States, in order to provide information on the methods of diagnosis used and the results of tests carried out in the Community;

(iv) retaining expertise on the relevant disease pathogen and other pertinent pathogens to enable rapid differential diagnosis;

(b) assist actively in the diagnosis of outbreaks of the relevant disease in Member States by receiving pathogen isolates for confirmatory diagnosis, characterisation and epizootic studies;
(c) facilitate the training or retraining of experts in laboratory diagnosis with a view to harmonising diagnostic techniques throughout the Community;
(d) collaborate, as regards methods of diagnosing animal diseases falling within their areas of competence, with the competent laboratories in third countries where those diseases are prevalent;
(e) collaborate with the relevant OIE reference laboratories with regard to exotic diseases listed in Part II of Annex IV under their responsibility;
(f) collate and forward information on exotic and endemic diseases, that are potentially emerging in Community aquaculture.

Work programme for 2015

Technical report

1. Coordination and training

1-1,1-2 Organise and prepare for the 19th Annual Workshop for the National Reference Laboratories for Fish Diseases (NRLs) and produce a report from the Annual Workshop

Organization of the 19th Annual Workshop

The 19th Annual Workshop of the National Reference Laboratories for Fish Diseases was held in Copenhagen, Denmark, 27th-28th May at the Auditorium of DTU Veterinary Institute in Bülowsvej 27, 1870 Frederiksberg C.

A total of 53 participants from 33 countries attended over the two days period, unfortunately 2 participants cancelled right before the meeting. There were five sessions with a total of 25 presentations, 3 of which were given by invited speakers, and a working group session.

The scientific program of the Annual Workshop was wide and covered many different topics of current interest.

The meeting was opened with the traditional session "Update on important fish diseases in Europe and their control", where participants from the Member States had the opportunity to present new findings from their respective countries.

Initially, an overview of the disease situation and surveillance in Europe 2014 was provided on the basis of the results obtained from the Survey & Diagnosis questionnaire.

Then the fish disease situation in Norway was presented; a detailed report in Norwegian is available at:
http://www.vetinst.no/nor/Publikasjoner/Fiskehelserapporten.
An English version will be available at:
http://www.vetinst.no/eng/Publications/Fish-Health-Report.

The results of a survey on the impact of fish diseases in the Mediterranean basin, achieved thanks to the collaboration with a panel of acknowledged fish pathologist experts, were presented.

The talks of the first session were completed by presenting an update on VHS and IHN control program which has been established and maintained in the Trento Region in Italy over the last 30 years, this latter talk included future perspectives and new initiatives.

The second half of the morning was allocated to an interactive activity, introduced for the first time during the Annual Workshop in 2014. Briefly, each participant was asked to consider the relevant infectious diseases for the 2 most important fish species farmed in his country. In this perspective, all participants received on beforehand 4 tables listing the most renowned pathogens for the most important farmed fish species in Europe. Before the WS each participant had the opportunity to interact with different stakeholders and assess impact on production, economy and legislative consequences for the different infectious diseases in 2014.

During the first part of this working group session, the activity was implemented at a country level, meaning that each participant was asked to rank the disease characterized by the higher impact in 2014. After this first level of investigation,

representatives of different macro-areas in Europe were grouped. The regions were Northern Europe, gathering the main Salmon producing countries, Eastern Europe focusing mainly on cyprinids and subsequently rainbow trout, Western Europe producing mainly rainbow trout and cyprinids and finally Southern Europe producing mainly the marine species European sea bass and gilthead sea bream and then rainbow trout. Experts had the possibility to discuss and describe the impact of each disease focusing on the 3 most important parameters. The first topic considered was the impact of a given disease on production, meaning the severity of losses in terms of mortalities, reduction of growth, etc. Then impact on the economy, indicating the cost of prevention (i.e. vaccination and biosecurity measures), treatment, reduced value of the product was considered. Finally consequences due to trade restrictions, national plans for control/eradication, suspension time after antibiotic treatment etc. Each group had to finalize its task by selecting and describing the most important diseases.
During the final part of the session a representative of each area described the agreed findings to the whole assembly.

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

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

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

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

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

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

In the evening a banquet dinner was held at Restaurant ***"The Italian".***

The second and last day was opened by the session on scientific research update; this fourth session faced several different topics covering molecular characterization of pathogens, a present and future core topic for all the laboratories involved in fish disease diagnosis, vaccination as a strategy to prevent infectious diseases and the establishment of experimental animal model to study infectious diseases. Focus was also given to new research initiatives dealing with fish diseases that recently started within the Horizon 2020 framework.

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

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

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

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

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

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

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

identifying microbial pathogens, benefits and challenges were described.

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

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

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

The final report of the 19th Annual Workshop is available on the web site
http://www.eurl-fish.eu/Activities/annual-meetings

1-3 Collect and report data on the fish diseases situation in EU, including all the listed non-exotic fish diseases given in Council Directive 2006/88/EC Annex IV Part 2

Survey and diagnosis of fish diseases in Europe in 2014

The Questionnaire on Surveillance and Diagnosis (S&D) which is collated annually is the only comprehensive overview of the disease situation in aquaculture in Europe. The information has been made available on the EURL web site (www.eurl-fish.eu), where all raw data can be obtained. The S&D have evolved and changed a bit over the years it comprise 3 parts:

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.

Production data from FIGIS

The data on the European aquaculture production were obtained from the FIGIS database. This database does not include information on the number and size of fish farms, and therefore these data had to be obtained directly in the questionnaire. In FIGIS only data from back to 2013 is available. Surprisingly and for the first time the total fish production in aquaculture in Europe did not increase in 2013. This is primarily reflected by the lower Atlantic salmon production, that might be due to the increase of the Chilean production or to a different reporting system in FIGIS. The Atlantic salmon production, however, still account for 1,43 mill ton against 1.5 mill ton in 2012, and is by far the largest contingency in Europe. The rainbow trout production has now passed the 400 000 t and increased with 12 000 t in 2013. The increase is however, primarily due to the increased production in Turkey! The carp production is still mainly in the Eastern part of Continental Europe and is very stable. Both the production of sea bream and especially sea bass also increased in the Mediterranean countries with a production of 142.000 t and 145.000 t, respectively. Among other fish species of interest are pike-perch (increase to 573t),

eel (decrease to 4.017t), sturgeon (increase to 5.584t), turbot (decrease from 12.676t to 9.891t), and halibut (decrease from 1.854t to 1.485t) the cod production have almost collapsed from 22.729t in 2009 to 4.252t in 2013. The production of cleaner fish for lice control e.g. lumpfish is increasing significantly but the total production is not that easy to retrieve due to the many species involved in this industry. Pike-perch have still not yet obtained the expected increase, but seem to be under way, while the sturgeon production is still on growing and more attention regarding health management might be given to this species.

Health categorization of fish farms:

Many countries provided very clear and correct answers but unfortunately a few more countries did not reply to the questionnaire when compared to the previous years. It is therefore still not possible to obtain a complete overview of the status of fish health categorization in Europe. There was however a significant increase in the reported number of categorized farms (From 8.505 in 2012 to 14.463 in 2013 for VHS and from 7.360 in 2012 to 11.962 in 2013 for KHV!) this was especially due to Germany who successfully included almost all their farms in categorized zones.

All most 3/4 of the authorised farms in Europe are situated in category III zones for VHS and 2/3 for IHN. For both diseases the remaining farms are situated in category I or II. Very few farms are placed in category V infected areas, and it is obvious that the diseases are very underreported. In all countries except Norway almost all salmonid farms are in Category I for ISA. Only very few carp farms are approved KHV free in Category I as almost all are placed in Category III or nor categorized.

There are several different views on how categorisation shall be performed, e.g. should VHS free marine rainbow trout farms be placed in Category III or I? According to a new proposal for adaptation by the EU Commission Isavirus HPR0 if detected in or in proximity of a farm, the farm can remain its Category I status. Some Member states do not include small registered APBs in the categorisation (e.g. hobby farms) but according to 2006/88/EC Annex III health categorisation comprise all APBs in the Member states, zones and compartments for each category. However, only fish species listed as susceptible for the given listed disease shall be included, i.e. no sea bass / sea bream / eel farms / pike.perch etc. for VHS, IHN, KHV and ISA categories)

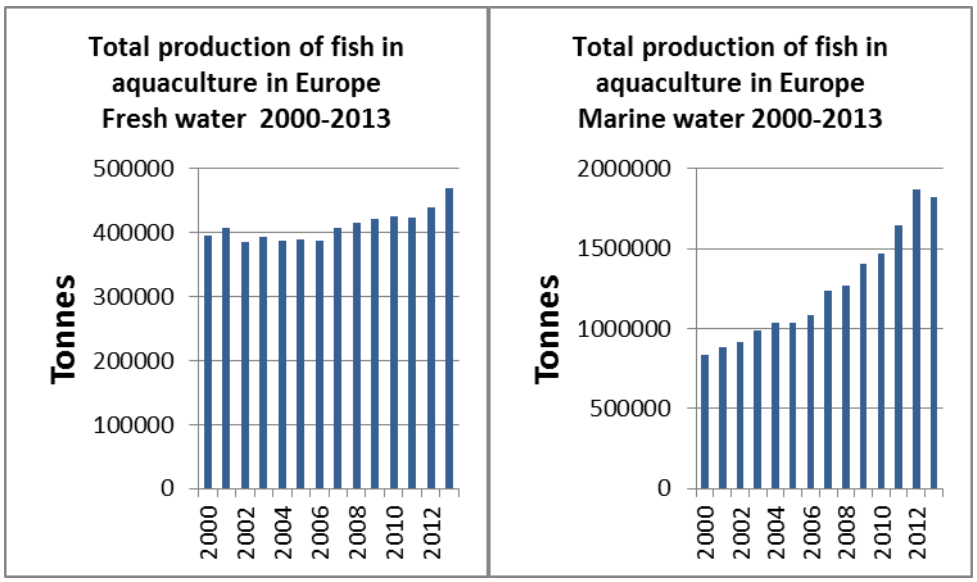
A new Animal Health Law is under preparation and revision and will include aquatic animals; in this connection the categorisation system might be simplified and be made more transparent.

Concerning the epidemiological data, obviously, there is still a severe underreporting of VHS, IHN and KHV in many countries. Only 52 and 54 of 8.896 farms are considered VHS or IHN infected, respectively, while 50 of 11.831 farms are considered KHV infected in the reporting countries. As in 2012 there were no ISA infected farms in Europe 31.12.2013! But there were a few outbreaks in Norway that have been contained.

Many countries have surveillance programmes for SVC (21 of 35 participating countries), BKD (17 of 35 countries), IPN (18 of 35 countries) and Gyrodactylus salaries (9 of 35 countries), for which they are seeking “additional guaranties” according to §42 in CD 2006/88/EC. The number of farms in the programmes varies from very few farms to many farms.

In northern European countries the most common problems are sea lice, pancreas disease, Amoebic gill disease in the salmon production, but in 2012 in addition

several countries reported finding of Winter Ulcer Disease in salmon caused by *Moritella viscosa.* In continental Europe it is primarily bacterial diseases like ERM and Aeromonas infections, AGD and RTFS, while problems in the Mediterranean countries are the same as in continental except for *Lactoccocosis* which is more common in Southern Europe and Nodavirus infection in mariculture which seem to play an increasing role.
There are very large differences between countries on how many samples are tested on cell cultures, ranging from < 100 to several thousands. The total number of samples examined by cell culture decreased with 8% since last year while PCR increased with 16% and is now more used than cell culture, e.g. Norway only tested 226 samples on cell cultures but 5000 samples by PCR, as they also skipped VHS/IHN surveillance by cell cultivation! The large number of PCR-tests conducted in some countries mostly reflects the KHV and ISA testing.



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

A summary of the results for 2014 is presented on our website:
www.eurl-fish.eu/Activities/survey_and_diagnosis.aspx

1-4 Facilitate and provide training in laboratory diagnosis. The yearly training courses in methods used for diagnosis of fish diseases will be offered at the EURL laboratory facilities. The courses will primarily be for training of staff from NRLs and the content will depend on request from participants.

Training

Training courses:

The EURL Fish is yearly offering 2 training courses in diagnostic techniques for identification of listed fish diseases and introduction to histopathology on fish diseases.
The training courses took place in Copenhagen at DTU National Veterinary Institute, Bülowsvej 27, 2700 Frederiksberg C Denmark, from October the 5th to the 15th, 2015. Two courses were prepared: the first one, with 10 trainees, was entitled "Methods for implementation of surveillance procedures for listed fish diseases" and took place from 5th to 9th October 2015.
The second course was entitled "Introduction to histopathology in fish diseases" and took place in Copenhagen 12th to 15th October 2015 with 14 participants. 1 person participated in both training courses.

The overall purpose of the training course was to provide an opportunity for the NRLs to send employees for training in techniques relevant when working with fish diseases.
Concerning the course on surveillance of listed fish diseases the first course the staff of the EURL provided this training, together with teachers from the Danish Veterinary and Food Administration
For the course focused on Histopathology, staff of EURL and DTU VET, in cooperation with NVI-Oslo and Aquapri DK, constitute the tutor team.
Knowledge-sharing and discussions between participants and teachers were important parts of the courses.

Course 1: Methods for implementation of surveillance procedures for listed fish diseases.

The 5-days course in "Methods for implementation of surveillance procedures for listed fish diseases" was primarily based on practical work (hands on) in combination with theoretical presentations.

Day 1 was dedicated to a fish farm inspection**.**
As procedure does not allow a visit to a fish farm after working in our laboratory it was decided to meet with the participants at the entrance of our institute and to drive to the FVO offices in Vejle, where we were received by Dr. Korsholm. After the training course introduction by Niels Jørgen Olesen and presentations on Danish surveillance plans for fish diseases held by Dr. Korsholm, the participants visited a rainbow trout farm, Vingsted Dambrug, approx 25 km from Vejle.
During the on-site visit, procedures for inspection and sample collection were demonstrated; participants were taught on how fish necropsy techniques are performed in the field. All participants had the opportunity to perform necropsy on diseased fish collected at the farm; they collected relevant samples and brought them back to the laboratory in Copenhagen for further examination.

On day 2 a detailed description of the course program was presented by N. Vendramin and discussed with the participants. After the introduction, a lecture on fish virology was given by prof. Niels Jørgen Olesen. The participants were then divided into two groups. As an assignment each participant had to process the samples collected in the fish farm and test it to rule out the presence of the listed disease VHS. Further on, participants were asked to investigate if other pathogens were present in the sample. The processing of fish samples collected the day before was demonstrated before the participants were asked to do it themselves.

Day 3 and 4 were replicated so that one group could follow the cell culture part one day and the Real-time PCR the day after and vice versa for the other group. With this organization it was possible for all the trainees to participate in all the practical activities that were demonstrated.
Every activity had a team of tutors in order to provide an effective support to the trainees. For the cell culture activities Niccolò Vendramin, Betina Lynnerup and Christina Flink Desler were assigned, while for the real-time PCR part, Susie Sommer Mikkelsen, Troels Secher Rundqvist and Didde Hedegaard Sørensen were the tutors.
The day dedicated to cell culture started with the demonstration of the procedure implemented in the laboratory to prepare diagnostic trays with BF-2 and EPC cell cultures. Subsequently all the participants prepared their own trays and inoculated the trays with the sample prepared the day before. Titration procedure was also demonstrated and a talk on titre calculation and cell susceptibility test was given. The afternoon was allocated to provide the participants with knowledge in reading different fish cell lines inoculated with different viral pathogens, highlighting features of different cytopathogenic effects (CPE). As practical assignment participants were asked to read titration plates previously prepared, calculate the titre of the virus inoculated, and describe and draw different CPE's on infected cell

cultures.

The day dedicated to real-Time PCR started with a theoretical presentation of the principles of PCR. The practical laboratory exercises started with participants being demonstrated how to purify RNA from their samples, later on each participant had the chance to process his own sample. Preparation of Mastermix was demonstrated, and one of the participants of the group would prepare the Mastermix, and mix it with the samples. Finally samples were loaded in the real-time PCR machine. While the machine was processing the samples, participants had time to have a session on troubleshooting and pitfalls in real-time PCR. At the end of the day, results of the analysis were collected and discussed.

Day 5 was allocated to finalize the course, discuss both results obtained by the participants and different methods for performing surveillance for listed fish diseases in their countries of origin. Finally a questionnaire for the course evaluation was given and participants asked to fill it anonymously.

The methods taught were primarily focused on the protocols given in EU legislation and on the OIE guidelines from the Manual of Aquatic Animal Diseases, and included how to select proper controls, the typical pitfalls, trouble shooting, etc. As get-together, a joint dinner the second evening was included.

Course 2: Introduction to histopathology in Fish Diseases.

The 4-days course in histopathology and immunochemical techniques was divided into theoretical lectures on relevant topics, practical exercises both in necropsy room and microscopy laboratory.

Day 1 started with introduction of course and practical information, each participant had the opportunity to present himself to the tutors and his fellows A practical demonstration on how to sample fish tissues and organs for proper histological examination followed, and each participant could practically try the technique. Lectures given by Ole Bendik Dahle from NVI-Oslo on the normal histology and artifacts followed after lunch break.
Day 2 was divided between practical observation of slides from confirmed case at the microscope and theoretical lectures focusing on general pathology. Once again Ole Bendik Dahle took the lead of the teaching supported by Tine Iburg from DTU VET.

The first part of day 3 was dedicated to lectures on Immunehistochemistry-IHC, the different phase of sample preparation for this staining technique and troubleshooting and pitfalls during the process were discussed this part of the program was conducted by Torsten Boutrup, previously employed at DTU Vet as fish pathologist and now employed in Aquapri, private company producing pikeperch. Right after lunch break a presentation on the In situ Hybridization technique, and its use in detection of animal pathogens. The lecture was given by Tim Kåre Jensen who has been using this technique on a large amount of pathogens, especially in pigs. The day was concluded with presentation and discussion of specific cases in an open forum where participants where welcome to comment the

images displayed on the screen.

Day 4, the last day of the course started directly at the microscopy room, diagnostic cases brought by participants were discussed and presented in an open forum, with superivision of tutors Ole Bendik Dahle and Tine Iburg.

After lunch, there was a last round of group work before all the participants met up again for a last discussion about the subjects that had been covered earlier in the day. At the end of the course the participants were provided with a questionnaire to fill in as part of the evaluation. Furthermore, the participants were invited to give their opinions on the course. All in all, the course was very well-received, and the distribution of practical and theoretical lessons seemed well balanced.

Through lectures, exercises, discussions, and knowledge exchange between participants, the knowledge on histopathology, and troubleshooting related to this technique was increased. There was much fruitful discussion among participants and teachers during the course, both during the lectures, the breaks and the theoretical exercises.

As get-together, an optional dinner event on day 2 was held.

Participant List

Name	Country	Course 1	Course 2
Areskog Marlene	Sweden		X
Peik Katrin	Estonia		X
Soares Silvia	Scotland		X
Nienius Darius	Latvia	X	x
Papp Melita	Hungary		X
Pupp Eszter	Hungary		X
Shanin Khalid	Scotland		X
Dumitrescu Florina	Bulgaria	X	
Fartat Laura	Romania	X	
Zele Diana	Slovenia	x	
Morrissey Teresa	Ireland	X	
Agnieska Belter	Poland	X	
Davidovich Nadav	Israel		X
Briadeau Tifanie	Basque Country		X
Kirova Svetlina	Bulgaria	X	
Luis Tiago	Portugal	X	
Clinton Morag	Scotland		X

Bolade Adeyemo	Nigeria		X
Arfara Stamatina	Greece		X
Stachnik Magdalena	Poland	X	
Meixner Doris	Chile		X
Tommy Berger	Norway		X
Volpe Enrico	Italy	X	

The final report is available on the EURL web site
www.eurl-fish.eu/Activities/traning

Master and PhD students:

Anna Luiza Farias Alencar, master's student at University of São Paulo, USP, has been enrolled for a six-month research internship starting in May, 2015. The research work was held at the EURL-Fish, DTU-Vet, and was finalized by a Final Report of the Scientific Research Project entitled "Experimental infection of Rainbow trout (Oncorhynchus mykiss) and Atlantic salmon (Salmo salar L.) with Piscine Reovirus and virus Y".

Ľubomír Pojezdal phd candidate from National Reference Laboratory for Viral Diseases of Fish in Brno-Czech Republic has been enrolled for 6 weeks internship to take part in validation process of IHNV RT-qPCR at the EURL Fish.

OIE collaboration:

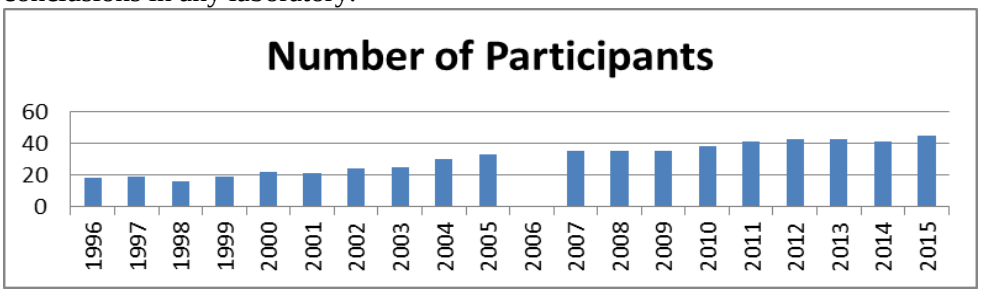
N.J. Olesen is designated expert of the OIE reference laboratory for VHS, and in this function had consultancies and requests worldwide concerning VHS. The OIE chapter on VHS and related diseases in the diagnostic manual for aquatic animal diseases is revised and updated by the OIE reference laboratory for VHS.
In this connection an OIE Twinning project have been established between the National Fishery Products Quality Management Service (NFQS) of the Republic of Korea, as the candidate institute, being the National Reference Laboratory (NRL) for fish diseases in Korea and DTU Vet. The candidate laboratory wants to improve the capabilities of performing its duties as the NRL for Viral Haemorrhagic Septicaemia (VHS). At the end of the twinning Project with the National Veterinary Institute, DTU, Denmark being the European Union Reference Laboratory for Fish Diseases (URL) and the OIE Reference Laboratory for VHS the NFSQ will apply for obtaining a status as OIE Reference Laboratory or OIE Collaborating Centre for fish diseases with focus on VHS for the Asian region. In this colaboration Dr Hyoung Jun Kim, Ph. D.National Fishery Peoducts Quality Management Service, RoK, visited us 8-10 July 2015

2. Proficiency tests

2-1 Prepare the Annual Inter-laboratory Proficiency Tests year 2014 for the NRLs. The tests include VHSV, IHNV, EHNV, ISAV, and KHV and in addition address other common viral pathogens in fish farming (IPNV, SVCV etc).

The inter-laboratory Proficiency Tests 2015

Since 1996, eighteen inter-laboratory proficiency tests (PTs) have been organised by the EURL. The number of participants has increased from 18 to 45. The goal of these tests is to harmonise diagnostic methods between national reference laboratories and to ensure that the examination of a given sample leads to the same conclusions in any laboratory.

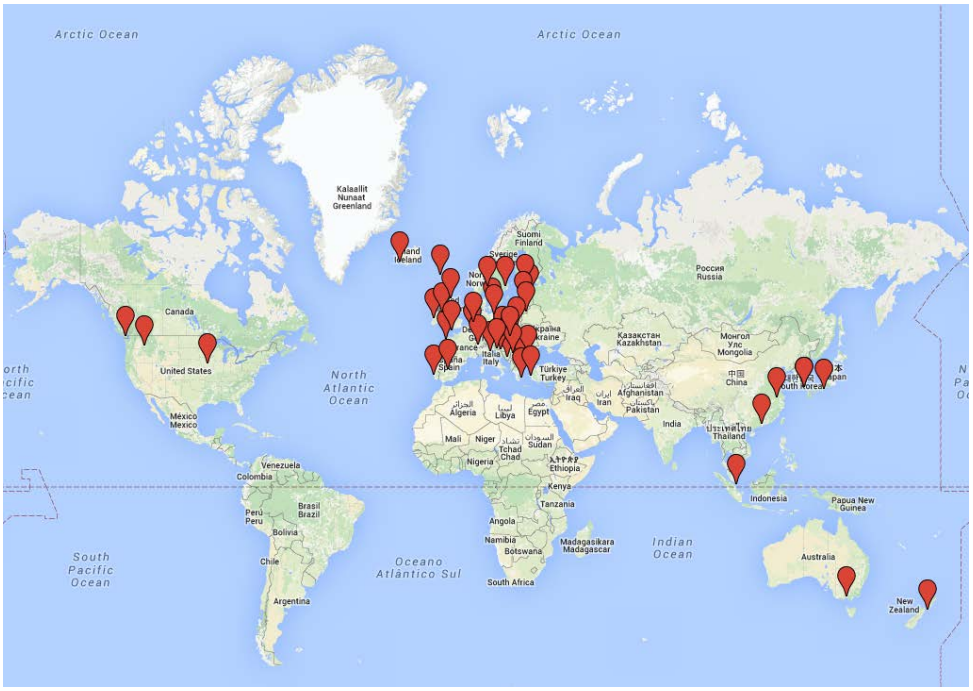


A comparative test of diagnostic procedures was provided by the European Union Reference Laboratory (EURL) for Fish Diseases. The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2).
PT1 was designed to primarily assess the identification of the fish viruses: viral haemorrhagic septicaemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV), epizootic haematopoietic necrosis virus (EHNV) or related rana-viruses by cell culture based methods. PT2 was structured with the aim of assessing the ability of participating laboratories to identify the fish pathogens: infectious salmon anaemia virus (ISAV), Salmon alphavirus (SAV) and Cyprinid herpesvirus 3 (CyHV-3) (otherwise known as *koi herpes virus* – KHV) by biomolecular methods (PCR based). 45 laboratories participated in PT1 while 44 participated in PT2 of which 34 participated in identifying SAV.
The tests were sent from the EURL in mid-September 2015.
Both PT1 and PT2 are accredited by DANAK under registration number 515 for proficiency testing according to the quality assurance standard DS/EN ISO/IEC 17043. This report covers both the results of PT1 and PT2.

PT1 consisted of five coded ampoules (I-V). These ampoules contained IHNV, VHSV alone and in a co-infection setting, ECV and Sterile medium (see table 1). The proficiency test was designed to primarily assess the ability of participating laboratories to identify any of the notifiable fish viruses VHSV and IHNV and to isolate any Rana-virus if present (Council Directive 2006/88/EC); furthermore the rana-virus isolate has to be further characterized in order to determine whether it is the listed pathogen EHNV or another representative of the rana-virus family and to identify 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 2015. 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 either method as mentioned in the IHN chapter of the 2013 version of the OIE manual on Aquatic Animal Diseases (Emmenegger et al. (2000), Diseases of Aquatic Organisms 40 (3), 163-176 or in Kurath et *al*. (2003) Journal of General Virology 84, 803-814) for IHNV. Laboratories were encouraged to submit all sequencing results used for genotyping the isolates.

PT2 consisted of four coded ampoules (VI-IX). One ampoule contained KHV, one contained SAV, one contained ISAV and one contained Sterile Medium, see table 9. The test was designed to primarily assess the ability of participating laboratories to identify the notifiable fish pathogens ISAV and KHV (listed in Council Directive 2006/88/EC) if present in the ampoules, bearing in mind that the test ampoules could also contain other pathogens. For 2015 the EURL has decided that the panel of pathogens to be investigated should be expanded including SAV-Salmonid Alpha virus. Since SAV is not a listed disease in the European legislation, all participants were free to decide if they would be testing for SAV or not. Each participant was asked to declare whether they would test or not. The EURL will then take care of calculating the score accordingly.
Participants were expected to use their normal PCR or real-time PCR methods for detection of the pathogens. Regarding SAV analysis, participants can refer to the OIE manual Chapter 2.3.5b. — Infection with salmonid alphavirus . 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.



NRL´s receiving the EURL PTs in 2015

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

Outcome of the Inter-laboratory Proficiency Tests 2015

Within one day, the tests were delivered to 22 participants; 14 more tests were delivered within the first week; 3 more within the first two weeks; 5 further within three weeks and the last test was delivered within 35 days.

Concluding remarks PT1:

The implementation of the task Inter-laboratory proficiency test 2015 posed some issues due to findings of participants for ampoule V. 49% of parcels were delivered by the shipping companies within 1 day after submission and 80% was delivered within 1 week. It was, however, unfortunate that six parcels were more than 2 weeks on the way and one of these was 35 days on the way before delivered to the laboratory primarily due to border controls.

This year ECV was included. 43 participants were able to identify Ranavirus of these laboratories 38 correctly identified ‘Ranavirus’ or ‘not EHNV’. 40 laboratories performed sequencing of these laboratories 35 correctly identified ‘ESV/ECV’.

In this report (Figures 5-8), all viral titres submitted by participants for each cell line and ampoule, respectively are compared to each other. In this way, the titres obtained by each laboratory are plotted in relation to the combined submitted data set and each participating laboratory can be able to compare the sensitivity of its cell lines to the sensitivity of those used by the other participants. We recommend

all participants to evaluate the sensitivity of the cells used in their laboratory in relation to the diagnostic purpose.

The EURL provides the annual proficiency test, collates the data and process the figures so that individual laboratories can see how they perform in relation to the other participants. It is up to the individual laboratory to assess if they perform according to their own expectations and standards. We will also take the opportunity to provide a comment to participants regarding submitted results if relevant. Furthermore we encourage all participants to contacts us with any questions concerning the test or any other diagnostic matters.

During interlaboratory proficiency test 2015, an unexpected result considering the content of Ampoule V was observed. The presence of a SVC-like rhabdovirus was confirmed in the ampoules in addition to the expected VHSV strain, DK-5151 + IHNV 32/87. The virus identified was Tench Rhabdovirus S64. On the background of the finding from the participant and the final confirmation performed at the EURL, the scoring system has been adjusted. This issue has been taken seriously into consideration from the EURL and managed both with the participants and DANAK the accreditation body that audit the QA system at DTU.
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Concluding remarks PT2:
This was the first time that the EURL provided a proficiency test on SAV identification. Considering that 33 laboratories participated (of which 32 correctly identified SAV in ampoule VII) this was regarded as a proper initiative that strengthen the diagnostic capacities of Europe in detecting emerging pathongens, and it will be included in coming years as well.
All 43 laboratories testing for KHV identified the virus in ampoule VI.
Out of the 33 laboratories there tested for SAV 32 laboratories identified SAV in ampoule VII.
Out of 44 laboratories 42 laboratories identified Not KHV or ISAV in ampoule VIII and there were only two false positive.
All 43 laboratories testing for ISAV identified the virus in ampoule IX, though one laboratory also wrongly identified KHV in ampoule IX.
It is an appreciated matter of fact that many laboratories are putting efforts in performing genetic characterization of the isolates through sequence analysis, as it is important that laboratories can discriminate between isolates as e.g. pathogenic ISAV strains with deletions in the HPR region and HPR0 strains.
Of the 27 laboratories sequencing the ISAV virus all found that the isolate was with deletion in segment 6 and thus not belong to HPR0.

The EURL provides the annual proficiency test, collates the data and process the figures so that individual laboratories can see how they fare in relation to the other participants. It is up to the individual laboratory to assess if they perform according to their own expectations and standards. We take the opportunity to provide comments to participants regarding submitted results if relevant. Furthermore we encourage all participants to contacts us with any questions concerning the test or any other diagnostic matters.

The results given in this report will be further presented and discussed at the 20th Annual Workshop of National Reference Laboratories for Fish Diseases to be held 31th of May-1st of June 2016 in Copenhagen, Denmark.

The final report is available on the EURL web site www.eurl-fish.eu/Activities/proficiency_tests

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

3. Reagents and products

Materials supplied by the EURL

On request, the EURL supplies material to other laboratories in Member States and Third Countries to aid in the diagnosis and characterisation of fish diseases. The number of laboratories receiving material and the specific material and number of units supplied by the EURL in 2015 are listed in Annex 3.

Further details of the supplied materials are listed in Annex 1

3-2. Production of diagnostic reagents against selected pathogens when necessary

Production of antisera

In 2015 no new productions of antisera were needed and our stocks of supernatants from hybridoma cells producing monoclonal antibodies were sufficient for the year.

3-3. Update and maintain a library of isolates of ISAV, VHSV and IHNV, KHV and EHNV and other relevant putative emerging fish pathogens.

Virus library

Isolates of the listed viruses VHSV, IHNV and KHV were received and stored in our library during 2015. In addition we received Ranavirus isolates from lumpfish in Iceland.

A panel of 24 IHNV isolates representing all known genotypes and subtypes was received from the OIE reference laboratory for IHNV USGR, Western Fisheries Research Centre, Seattle, (Dr. Gael Kurath), propagated and aliquoted for further distribution to the EU NRL network. All isolates are full genome sequenced and provide a very useful tool for further research.

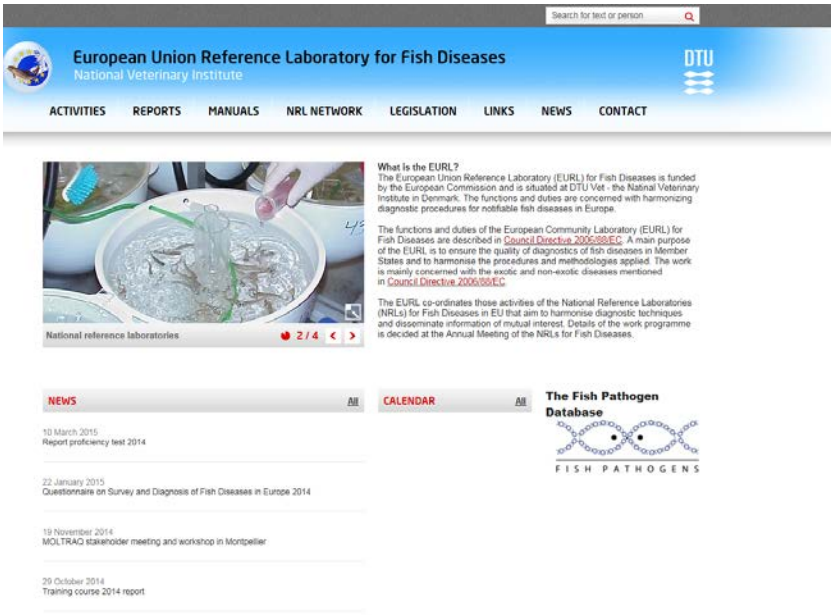
Further details of the received materials are listed in Annex 2

4. Scientific advice and activities

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

Update the webpage of the EURL

The EURL website (www.eurl-fish.eu) is a notice board, where NRLs and other interested parties can access relevant information and previous reports concerning the activities coordinated by the EURL and relevant upcoming events in the Union.



The diagnostic manuals for VHS, IHN, ISA, KHV, EHN disease and EUS have been updated and uploaded on the web page.
Furthermore, reports of the EURL, e.g. of the results of the proficiency test, the Annual Meeting of the NRLs, result of questionnaire on "Survey & Diagnosis", Training courses etc. are launched at the web page immediately after release.

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

Diagnostic manuals

During 2015 the Commission Implementing Decision (EU) 2015/1554 of 11 September 2015 laying down rules for the application of Directive 2006/88/EC as regards requirements for surveillance and diagnostic methods was finally adopted following intensive correspondence between the Member States, the Commission and the EURLs. Several compromises were accepted to satisfy demands from the MS and keeping scientific level and justification at an acceptable level.
The final versions of the surveillance, sampling and diagnostic procedures for the listed fish diseases are uploaded on the EURL Fish website for easy and comprehensive access to the details in the Decision.
The decision only comprise the non-exotic diseases listed in the directive 2006/88/EC, therefore a non-approved version of the sampling and diagnostic

procedures for the exotic disease Enzootic Hematopoietic Necrosis (EHN) are included as are the methods for detection of EUS which was delisted previously but were the diagnostic procedures are still in force.The new Decision replace the Commission Decision 2001/183/EC on VHS and IHN and Commission Decision 2003/466/EC on ISA.

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.

Fishreflabnet:

An e-mail group was created in 2012: VET-EURL with approximately 100 colleagues subscribing. Official communication and updates of interest to the scientific community are delivered periodically.
Furthermore this tool is used for newsletters, scientific updates and announcements from the EURL Fish like announcements and invitations for the Annual Workshop or publication of content in the ampoules from the proficiency test or on the final Inter-laboratory Proficiency test report. In addition the e-mail group is used for announcing other workshops, training courses and conferences and new publications of interest for the NRL Fish network

4-4 Pathogen characterization: Identify and characterise selected isolates of listed viruses (pathogenicity testing in vivo and in-vitro, serological and genetic characterisation).

Studies conducted on pathogen characterization:

Almost 300 VHSV isolates from both marine and freshwater rainbow trout farms, spanning from 1978-2009, including all known isolates in Denmark from 1993 onward, were selected for analysis in the largest study of Danish VHSV isolates to date. The full-length G-gene was sequenced for all new isolates. Genetic data and epidemiological information have been used to infer phylogenetic trees and phylogeographic models for viral spread to analyse the relationship between VHS outbreaks in Denmark and to understand the diffusive dynamics of the disease over a historical period, as well as the effectiveness of the containment and eradication programme.

Identification and characterisation of selected virus isolates:
In 2015 a number of virus isolates, sera and other reagents were received for further characterisation at the EURL and for storing in our virus library as shown in the table beneath.

Member States and countries outside EU		
Material received	**Laboratories**	**Units**
Diagnostic material for virology	8	94 samples
Diagnostic material for PCR	3	24 samples
Diagnostic material for bacteriology	2	20 samples
PCR control material	1	2 sample
MAb/PAb	2	7 samples
Other material	1	3 samples

Further details are listed in Annex 2

Below is listed samples, isolates and reagents received for identification, characterization and update of the virus library and diagnostic procedures applied for the relevant cases:

Sakana del Peru S.A., Peru (Paita): Sardines, kidney for VHSV examination. All samples tested negative by real time RT-PCR (DTU-VET 15-2824)

National Veterinary Institute, Norway (Helena Hauge): Blood from PRV-like infected rainbow trout received for assessment of risk of a putative new emerging diseases in rainbow trout farming in a collaboration with NVI, Norway (DTU-VET 15-4566)

Veterinary Institute, Croatia (Snjezana Zrncic): Sea Bass. Brain for bacteriological examination. *Vibrio Anguillarum* and *Vibrio Harvey* isolated (DTU-VET 15-7075).

USGR Western Fisheries Research Center, USA (James Winton): G24 reference Set, a panel of 24 IHNV isolates to be delivered to European partners for validation of diagnostic methods of IHNV viruses have been propagated, aliquoted and stored in freezer.(DTU-VET 15-7386)

NMBU- Vet School Norway (Espen Rimstad): polyclonal antibody anti PRV (15-8187)

Institute for experimental pathology, Iceland (Sigridur Gudmundsdottir): Supernatant of cell culture from Lumpfish infected with non-identified virus. A new ranavirus is identified and genetically characterized (DTU-VET 15-8086)

National Vet. Service , Bulgaria (Vanya Chikova): Cell culture supernatant and tissue from moribund carl. No virus detected (DTU-VET 15-9063)

Institute of Veterinary Medicine, Serbia (Vladimir Radosavljevic): Carp Tissues for KHV confirmation 10 samples. 1 sample confirmed positive for KHV, and 4 for Cyprinid Herpesvirus (DTU-VET 15-10834)

Veterinary Research Institute, Czech Republic (Tomas Vesely): Mab anti – SVCV G2E1 (DTU-VET 15795)

National Veterinary Institute, Sweden (Eva Blomkvist): Cell supernatant from eel for detection of Herpes Virus Anguilla, no virus has been detected with PCR. (DTU-VET 15-18128)

Istituto Zooprofilattico Sperimentale delle Venezie IZSVe , Italy (Anna Toffan) :
2 VHSV strains for confirmation, further genetic characterization and cooperative project with Aarhus university (field vaccination trial) (DTU VET 15-18464)
3 IHNV strains or confirmation, further genetic characterization and cooperative

project with Aarhus university (field vaccination trial) (DTU VET 15-18466)
1 Infected cell culture supernatant suspected for co-infection. VHSV and Perch Rhabdovirus identified (DTU VET 15-18465)

Institute for experimental pathology, Iceland (Sigridur Gudmundsdottir): Supernatant of cell culture from Lumpfish infected with VHSV for confirmation. Virus detected and further genetically characterized. Virus strain used in risk assessment in vivo infectious trial with Atlantic Salmon and Rainbow trout (DTU-VET 15-19852)

University of Bern, Switzerland (Thomas Wahli) 4 IHNV infected cell culture from rainbow trout for cooperative research project (DTU-VET 15-21831)

Central Veterinary Institute-CVI Of Wageningen UR, The Netherlands (Olga Haenen) Carp infected tissues for Carp Edema Virus Positive control material (DTU-VET 15-22608)

University of Ljubljana, Slovenia (Vlasta Jencic) Kidney Tissue of 150 rainbow trout, grouped in 30 pools for BKD testing. All samples tested negative (DTU-VET 16-4569)

Iran Veterinary Organization (Nastaran Shahbazian). 11 cell culture supernatants for virological examination – IHNV detected in 5 of 11 samples, and IPNV in 3 samples, no CPE observed in other 3 samples. 14 samples received for examination for VHSV. VHSV detected in 12 of 14 samples. Phylogenic analysis showed European origin of the VHSV and IHNV isolates, but also indicated that the virus have been present for a longer time in Iran

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

The pathogen database www.fishpathogens.eu

A large number of isolates were inserted in the database- now comprising 755 VHSV records, 92 records of IHNV and 108 betanoda records. Additionally, more than 400 VHSV isolates have been added, but are waiting to be released pending publication of papers. A new article "Fishpathogens.eu/noda: a free and handy online platform for Betanodavirus targeted research and data sharing" concerning the betanoda database has been published in Journal of Fish Diseases (J Fish Dis. 2015 Aug;38(8):755-60).
A new database for SAV is being established in collaboration with the Norwegian Veterinary institute.
Furthermore, a number of bugs have been corrected.

4-6 Perform molecular epidemiology analysis to improve knowledge on diseases spreading mechanisms of viral pathogens.

Molecular epidemiology analysis.

A paper entitled entitled "Phylogeny of the Viral Hemorrhagic Septicemia Virus in European Aquaculture" have been submitted to PLOS ONE with the following author list: Michael Cieslak; Susie S Mikkelsen; Helle F Skall; Marine Baud; Nicolas Diserens; Marc Y Engelsma; Olga LM Haenen; Shirin Mousakhani; Valentina Panzarin; Thomas Wahli; Niels J Olesen; Heike Schütze. We investigated this topic using the largest VHSV *Ia*-isolate dataset ever compiled, comprising 626 complete *G* gene sequences, 422 of which were *Ia* isolates identified in this study. The sequences come from 11 European countries and cover the period 1971–2015. Based on this dataset, we documented the extensive spread of the *Ia* population and the strong mixing of *Ia* isolates, apparently by means of the Europe-wide trout trade. Another study: “Molecular Tracing of Viral Haemorrhagic Septicaemia Outbreaks in Denmark” where almost 300 Danish VHSV isolates from both marine and freshwater rainbow trout farms, spanning from 1978-2009, including all known isolates from 1993 onward, were selected for analysis in the largest study of Danish VHSV isolates to date. The full-length G-gene was sequenced for all new isolates. Genetic data and epidemiological information have been used to infer phylogenetic trees and phylogeographic models for viral spread to analyse the relationship between VHS outbreaks in Denmark and to understand the diffusive dynamics of the disease over a historical period, as well as the effectiveness of the containment and eradication program

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4-7 Assessment and standardization of real-time PCR tests for the diagnosis, identification and typing of emerging and listed non-exotic and exotic fish diseases

Real-time PCR

A standardization and validation of the real-time RT-PCR developed by Purcell et al.: "Universal reverse-transcriptase real-time PCR for infectious hematopoietic necrosis virus (IHNV)" was initiated during the internship of Ľubomír Pojezdal. The method is being translated and validated to a one-step procedure which is more convenient for use as a tool for surveillance of IHNV. The validation will be finalized and published in 2016. A real-time RT-PCR for detection of Piscine reovirus-like virus from rainbow trout have been implemented and optimized in addition to a PRV real-time RT-PCR validated during the internship of Anna Alencar.

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

Emerging diseases:

The following studies on emerging diseases have been conducted in 2015:

CEV 20 participants from 11 countries attended a 2-day meeting organized by the EURL in collaboration with CVI, NL, in order to assess the risk, diagnostic methods and implications for the carp industry of carp edema virus infections.

Studies have been ongoing on detection of RLO- Rickettsia like organism in Sea bass in collaboration with the Veterinary Institute, Croatia (Snjezana Zrncic)

Wild Atlantic salmon was screened for the presence of PRV, 4 of 181 salmon were positive while an addition of 5 salmon were suspected positive.

PRV and IHNV co-infection studies. In order to assess the risk of a potential IHNV infection in PRV infected Atlantic Salmon (*Salmo Salar L.*), and the possible interaction between PRV and IHNV co infection an infection trial was conducted in salmon, with a setting for continuous sampling at several time points and another setting for mortality and disease monitoring. A cohabitation model was chosen for the PRV infection and bath immersion in infected water for IHNV.

VirusY or (PRV-2) is a virus detected in connection with disease outbreaks in rainbow trout in Norway. In close collaboration with the Veterinary Institute and NMBU in Norway an experimental infection trial with VirusY was conducted on rainbow trout and Atlantic salmon, respectively with continuous sampling at several time points and kinetic studies on different tissues. A cohabitation model was chosen.

5. Missions

Missions to relevant laboratories

5-1 Missions: Organizing missions to relevant laboratories. Missions focus on NRLs where on-site communication would be beneficial. As collaboration with

In 2015 three missions were conducted.

Visit in Korea.

Prof. N.J. Olesen participated in a TAIEX mission on export of live olive flounder to the European Union Ref: Exp 60429, 22-27 November 2015. During this visit

NRLs in 3rd countries from where EU is importing large amount of fish

collateral meetings and visits to the National reference laboratory for Fish Diseases in Korea was conducted and recommendations given for diagnostic procedures

Visit in Iran.

The Iranian Veterinary Organization invited prof. N. J. Olesen for a 1 week workshop and study visit to the national laboratory in Tehran and to a regional laboratory in Sharecord. All facilities were inspected and a number of advices were given for improving the facilities and sampling and diagnostic procedures as well as control measures. Iran has a very significant production of rainbow trout (between 100 and 150.000 ton/year) and has encountered very serious problems with both IHN and VHS in the recent years. Both viruses seem to have been introduced to Iran through trade from Europe. As an outcome it was agreed that IVO in future will participate in the Annual inter-laboratory proficiency tests organised by the EURL

Visit in Iceland

The National Reference Laboratory for Fish Diseases (NRL) in Iceland is located at the Institute of experimental pathology at Keldur. The Institute was visited from the 26th-27th of November 2015 by Niccolò Vendramin and Teena Vendel Klinge, coordinator of the EURL. A program for the visit was set up and a number of persons from the Icelandic authorities and laboratory met with the EURL representative. The report from the mission describes findings, comments and recommendations made by the EURL representative.

The report is enclosed as Annex 3

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.

International meetings organized.

Contact with colleagues from other laboratories is a channel for exchange of information in the field of fish diseases, and an opportunity to keep abreast with new developments in the field. Of special interest are of course the activities relating to VHS, IHN, KHV, ISA and EHN, but a number of emerging disease are coming up and need attention as well. Scientists at the EURL participated in the following international meetings and conferences in 2015:

CEV-Workshop

The Carp Edema Virus- CEV Workshop was held in Copenhagen, Denmark, 12th-13th January 2015 at the premises of DTU Veterinary Institute in Bülowsvej 27, 1870 Frederiksberg C. A total of 20 participants from 11 countries attended the meeting over the two days period. The workshop combined different single oral presentations and sessions with general discussions.

The workshop was organized and held due to the increasing amount of diagnostic cases where CEV was detected in diseased cyprinids (both Koi and common carp).

The primary aim of the workshop was to share knowledge, diagnostic protocols and material among participants and evaluate different strategies on how to tackle this issue. During the first session of the first day representatives of all countries participating in the workshop described their experience and the cases where fish poxvirus was detected. Subsequently diagnostic procedures available for the detection of this pathogen were described and compared. In the evening a banquet dinner was held at Restaurant “Cassiopeia”. During the second and last day a

common strategy on how to tackle this pathogen with research project was addressed, looking into funding opportunities and cooperative activities.

Name	Institution
Sven Bergmann	FLI-Germany
Heike Schütze	FLI-Germany
Laurent Bigarré	Anses
Mikolaj Adamek	University of Veterinary Medicine in Hanover
Verena Jung-Schroers	University of Veterinary Medicine in Hanover
Tomáš Veselý	Veterinary Research Institute, Brno, Czech Republic
Olga Haenen	CVI, part of WUR, Lelystad
Thomas Waltzek	University of Florida
Mansour El Matbouli	University of Veterinary Medicine Vienna
Veronika Piačková	University of South Bohemia
Keith Way	CEFAS
David Stone	CEFAS
Marek Matras	Piwet_Poland
Mona Gjessing	NVI
Ole B. Dale	NVI
Anna Toffan	IZSVE
Miriam Abbadi	IZSVE
Niels Jørgen Olesen	DTU-VET
Susie Sommer Mikkelsen	DTU-VET
Niccoló Vendramin	DTU-VET

Further information can be retrieved at http://www.eurl-fish.eu/Reports

Participation at international conferences and meetings

MOLTRAQ-workshop Montpellier 26th-30th January
PARTICIPANTS: Niels Jørgen Olesen, Susie Sommer Mikkelsen

19th Annual Workshop of the National Reference Laboratories for Fish Disease. Copenhagen, 27th-28th May, 2015.
PARTICIPANTS: Niels Jørgen Olesen, Lone Madsen Tine Iburg, Susie Sommer Mikkelsen, Niccoló Vendramin

DAFINET AND PROFISH WORKSHOP PATHOGEN – HOST INTERACTIONS AND VACCINE EFFECTS a 2 day Workshop and Ph.D. course, University of Copenhagen, Denmark, November 17th, 18th 2015
PARTICIPANTS: Niels Jørgen Olesen, Lone Madsen, Niccoló Vendramin

17th International Conference on Diseases of Fish and Shellfish September 7-11 2015. Las Palmas de Gran Canaria . Participants: Niels Jørgen Olesen, Lone Madsen, Susie Sommer Mikkelsen, Tine Iburg, Niccoló Vendramin

Peer reviewed publications
Presentations and posters

Presentations and posters

Articles published in peer-reviewed journals:

Mikkelsen, S. S., Panzarin, V., Jonstrup, S. P., Bigarré, L., Baud, M., Gray, T., Agapow, P.-M. and Olesen, N. J. (2015), Fishpathogens.eu/noda: a free and handy online platform for Betanodavirus targeted research and data sharing. Journal of Fish Diseases. doi: 10.1111/jfd.12378

O.L.M. Haenen, H. Schuetze, M. Cieslak, S. Oldenburg, M.A.H. Spierenburg, I. Roozenburg-Hengst, M. Voorbergen-Laarman, M.Y. Engelsma and N.J. Olesen (2016) First evidence of Infectious Hematopoietic Necrosis Virus (IHNV) in the Netherlands. Journal of Fish Diseases doi:10.1111/jfd.12434

Takafumi Ito, Jun Kurita, Koh-ichiro Mori, Helle Frank Skall, Niels Lorenzen, Katja Einer-Jensen, Niccolò Vendramin, and Niels Jørgen Olesen (2015) Molecular features of low and high pathogenic clones of viral hemorrhagic septicemia virus (VHSV) Ib isolates from rainbow trout *Oncorhynchus mykiss*: virulence variability within genotype Ib isolate.

Takafumi Ito, Jun Kurita, Koh-ichiro Mori, Niels J Olesen (2016) Virulence of viral haemorrhagic septicaemia virus (VHSV) genotype III in rainbow trout. Vet Res 47:4

Helle Frank Skall, Claus Jørgensen, Niels Jørgen Olesen (2015) Evaluation of the effect of percolation and NaCl solutions on viral haemorrhagic septicaemia virus (VHSV) under experimental conditions. Aquaculture, Volume 448, Pages 507–511

Ian A. Gardner, Richard J. Whittington, Charles G.B. Caraguel, Paul Hick, Nicholas J. G. Moody, Serge Corbeil, Kyle A. Garver, Janet V. Warg, Isabelle Arzul, Maureen K. Purcell, Mark St. J. Crane, Thomas B. Waltzek, Niels J. Olesen, Alicia Gallardo Lagno Recommended reporting standards for test accuracy studies of infectious diseases of finfish, amphibians, molluscs and crustaceans: the STRADAS-aquatic checklist. DAO

Jones SRM, Bruno DW, **Madsen L** & Peeler EJ (2015). Disease management mitigates risk of pathogen transmission from maricultured salmonids. *Aquaculture Environment Interactions* **6**, 119-134

Castillo D, Christiansen RH, Dalsgaard I, **Madsen L** & Middelboe M (2015). Bacteriophage resistance mechanisms in the fish pathogen *Flavobacterium psychrophilum*: linking genomic mutations to changes in bacterial virulence factors. *Applied and Environmental Microbiology* **81**:3, 1157-1167

Abstracts, proceedings and reports at international meetings

19th Annual Workshop of the National Reference Laboratories for Fish Diseases, Copenhagen, Denmark, May 27-28 2015 with the following presentations:

1: Overview of disease situation in Europe, Niels Jørgen Olesen
2: Update on the disease situation in aquatic organisms in the Mediterranean Niccolò Vendramin
3: Diagnostic manuals. Niels Jørgen Olesen
4: Molecular Tracing of aquatic viruses Moltraq. Susie Sommer Mikkelsen
5: Parafish - Advanced Tools and Research Strategies for Parasite Control in European farmed fish. Niccoló Vendramin

17th EAFP Conference, Gran Canarias, September 2015 (European Association of fish pathologists)

Detection of Neutralizing Antibodies Specific to Koi Herpesvirus (KHV) by Serum Neutralization Test Cabon J., Louboutin L., Castric J., Bergmann S.M., Bovo G., Matras M., Haenen O.5, N.J. Olesen, Morin T.

Workshop: Fish health in Mediterranean Aquaculture, past mistakes and future challenges Niccoló Vendramin, Snjezana Zrncic, Francesc Padros and Drazen Oraic

Serological Diagnostics For Fish Diseases –The Underestimated Tool. S. M. Bergmann, M. Matras, N. J. Olesen, Th. Morin and J. Kempter

Genetics of VHSV in Europe. M. Cieslak, M. Baud, N. Diserens, M. Engelsma, O. Haenen, S. Mousakhani, S. S.Mikkelsen, N.J. Olesen, V. Panzarin, H.F. Skall, T.Wahli, H.Schütze

RLO infection in European sea bass – an emerging disease in Mediterranean Mariculture S. Zrncic ,N. Vendramin, T. S. Boutrup, M. Boye, M. S. Bruun D. Brnic,D. Oraic

Molecular tracing of VHS in Denmark. S.S. Mikkelsen, H. Schuetze, H. Korsholm, B. B. Jensen , M.S. Bruun, N.J.Olesen

Mass Mortalities in Baltic Sea Eelpout (Zoarces Viviparous) caused by a new Rhabdovirus. M. Areskog, E Blomkvist, A Alfjorden, M Hakhverdyan, T S Boutrup, H Ahola, F Ljunghager, Å Hagström, N J Olesen, M Juremalm, M Leijon, J-F Valarcher, C Axén.

Characterization of Eelpout Rhabdovirus (Erv) on Cell Culture. E Blomkvist*1, A Alfjorden1, M. Hakhverdyan, T S Boutrup, H Ahola, F Ljunghager, Å Hagström, N J Olesen, M Juremalm, M Leijon, J-F Valarcher, C. Axén

Complete Genome Sequence of Eelpout Rhabdovirus (Erv) Identified By Deep Sequencing of Viral RNA M Hakhverdyan, M Areskog, E Blomkvist, A Alfjorden, T S Boutrup, H Ahola, F Ljunghager, Å Hagström, N J Olesen, M Juremalm, J-F Valarcher, C Axén, M Leijon

Inactivation of VHSV by Infiltration and Salt under Experimental Conditions. H.F. Skall, C. Jørgensen & N. J. Olesen

Viral Haemorrhagic Septicaemia Virus (VHSV): On the Search for Determinants Important for Virulence in Rainbow Trout *Oncorhynchus Mykiss*. N. J. Olesen, H.F. Skall, J. Kurita, K. Mori and T. Ito

Inter-laboratory proficiency test on notifiable fish diseases: a tool to strengthen diagnostic capacities for viral fish diseases. N. Vendramin, S.S. Mikkelsen, N.J. Olesen

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VHS virus – present situation. Niels Jørgen Olesen and Helle Frank Skall
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International scientific collaborative studies

Participation in international scientific collaborative studies

MOLTRAQ: Molecular tracing of aquatic animal diseases

The group was partner in the FP7 EMIDA-ERA Net project MOLTRAQ and work package leader of WP6: Dissemination and exploitation.

The purpose of the project was to increase knowledge on transmission, prevention and control of viral diseases in aquaculture and to develop a generic approach to viral disease control by using information on epidemiological and phylogenetic attributes from several important aquatic animal viruses.

We provide isolates from our large collection for sequencing at other institutes and we also sequenced a large number of isolates ourselves. Data was used to generate phylogenetic trees and to identify important factors in the evolution and spreading of viruses.

Furthermore, all isolate and genetic information on VHSV and IHNV is uploaded to the EURL database for fish pathogens: www.fishpathogens.eu.

Further information is available at the public project website www.moltraq.wordpress.com.

AQUAEXCEL^{2020}

The DTU Fish diseases group is partner in AQUAEXCEL^{2020}.The objectives of AQUAEXCEL^{2020} are to provide a wider and more efficient access to, and use of, the aquaculture research infrastructures existing in the EU. AQUAEXCEL2020 is a key vehicle in the improvement of aquaculture research practices. It will lead to a better management of animal experiments for research according to the 3 R's. One major feature of AQUAEXCEL2020 is its **Transnational access (TNA) program**, allowing external teams to access the partners' infrastructures via submission of research proposals, which are funded or not based on evaluation by an independent selection panel. Detailed information is available at www.aquaexcel.eu

ParaFishControl.

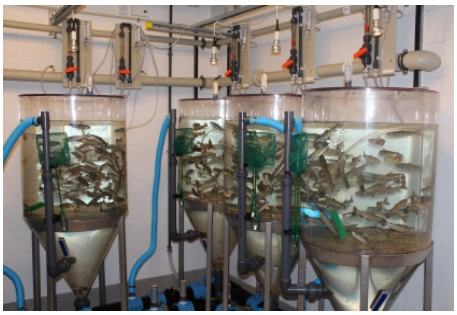
DTU is partner in the H2020 project ParaFishControl under the call "sustainable food security". The aim of the project is to 1) generate new knowledge on the most important parasites in fish farming including genome analysis, life cycles, and host parasite interactions especially in order to improve prophylaxis 2) assess the impact of transfer of parasites between farmed and wild fish. 3) develop an array of new prophylactic measures, including vaccines and functional feed. 4) develope speific and sensitive diagnostic tools 5) risk assessments, 6) identify zoonotic risks 7) develop catalogue on GMP

The main objectives for DTU is:

1. Validation of diagnostic procedures for detection of parasites based on qPCR, ISH and IHC, by e.g. inter-laboratory proficiency tests.

2. Participate in development of "point of care" diagnosis

3. Participate in publishing diagnostic protocols and standards.
4. Optimization of use of cleaner fish for controlling ectoparasittes.
5. Treatment and management of infected fish farms.



Annex 1 Reagents supplied by the EURL-Fish in 2015

Country	Name	Institute	Date of receipt	Material	Amount	Protocol No.
Peru	Paita	SAKANA DEL PERU S.A.	09.02.2015	Mid-kidney sardine tissue for VHS examination	6 tubes	2015 - 2824
Norway	Helena Hauge	National Veterinary Institute	10.03.2015	Atlantic salmon blood intected with Y-virus (material for infection trial)		2015 - 4566
Croatia	Snjezana Zrncic	Veterinary Institute	23.04.2015	Seabass, brain for bacteriology	5	2015-7075
USA	James R Winton	USGR, Western Fisheries Research Center	30.04.2015	Cell culture fluid with different isolates of IHNV for IHNV panel (G24 Reference set)	24 tubes	2015-7386
Norway	Espen Rimstad	NMBU	18.05.2015	Mab PRV from rabbit	2	2015-8187
Iceland	Sigridur Gudmundsdottir	Institute for Experimental Pathology	13.05.2015	Supernatant of cell culture from Lumpfisk (kidney/heart) infected with unidentified virus for diagnostic testing.	6 vials	2015-8086
Bulgaria		National Veterinary Service	28.05.2015	Cell culture supernatant from Ulcer, Papilloma and Xondahat for identification	3 tubes	2015-9063
Serbia	Vladimir Radosavljevic	Institute of Veterinary Medicine	24.06.2015 and 06.07.2015	Carp tissue homogenated gills and kidney for KHV confirmation	2 tubes + 8 tubes	2015-10834
Czech Republic	Tomas Vesely	Veterinary Research Institute	02.09.2015	Mab SVCV-G 2E1	5 tubes	2015-15795
Iran	Nastaran Shahbazian	Iran Veterinary Organization	28.09.2015	Tissue material, cell culture supernatant and tissue in ethanol	25 tubes	2015-17753
Sweden	Eva Blomkvist	National Veterinary Institute	30.09.2015	Eel tissue and supernatants for identification	7 tubes	2015-18128

Country	Name	Institute	Date of receipt	Material	Amount	Protocol No.
Italy	Anna Toffan	Istituto Zooprofilattico Sperimentale delle Venezie IZSVe	07.10.2015	Rainbow trout, Supernatant for diagnostic confirmation and sequencing	2 tubes	2015-18464
				Rainbow trout, Supernatant for diagnostic confirmation/identification	1 tubes	2015-18465
				Distinct IHNV viral strains for forwarding	3 tubes	2015-18466
Iceland	Sigridur Gudmundsdottir	Institute for Experimental Pathology	27.10.2015	Supernatant of EPC cell culture from Lumpfisk (kidney/heart) infected with unidentified virus for diagnostic testing.	4 tubes	2015-19852
Switzerland	Thomas Wahli	University of Bern	18.11.2015	Rainbow trout. Cell culture supernantant. IHNV isolates.	4 tubes	2015-21831
The Netherlands	Olga L.M. Haenen	Central Veterinary Institute (CVI) of Wageningen UR	26.11.2015	Carp, supernatant, controls for CEV PCR assay	2 tubes	2015-22608
Greece	Georgios Spiliopoulus	Andromeda Group	17.12.2015	Whole fish for virological and bacterial examination	15	2015-23618
Slovenia	Prof. Vlasta Jencic	University of Ljubljana, Veterinary Faculty.	23.12.2015	Rainbow trout, kidney tissue for BKD examination	30 tubes	2016-456

Annex 2 Reagents received in 2015

Country	Name	Institute	Date of receipt	Material	Amount	Protocol No.
Peru	Paita	SAKANA DEL PERU S.A.	09.02.2015	Mid-kidney sardine tissue for VHS examination	6 tubes	2015 - 2824
Norway	Helena Hauge	National Veterinary Institute	10.03.2015	Atlantic salmon blood intected with Y-virus (material for infection trial)		2015 - 4566
Croatia	Snjezana Zrncic	Veterinary Institute	23.04.2015	Seabass, brain for bacteriology	5	2015-7075
USA	James R Winton	USGR, Western Fisheries Research Center	30.04.2015	Cell culture fluid with different isolates of IHNV for IHNV panel (G24 Reference set)	24 tubes	2015-7386
Norway	Espen Rimstad	NMBU	18.05.2015	Mab PRV from rabbit	2	2015-8187
Iceland	Sigridur Gudmundsdottir	Institute for Experimenta l Pathology	13.05.2015	Supernatant of cell culture from Lumpfisk (kidney/heart) infected with unidentified virus for diagnostic testing.	6 vials	2015-8086
Bulgaria		National Veterinary Service	28.05.2015	Cell culture supernatant from Ulcer, Papilloma and Xondahat for identification	3 tubes	2015-9063
Serbia	Vladimir Radosavljevic	Institute of Veterinary Medicine	24.06.2015 and 06.07.2015	Carp tissue homogenated gills and kidney for KHV confirmation	2 tubes + 8 tubes	2015-10834
Czech Republic	Tomas Vesely	Veterinary Research Institute	02.09.2015	Mab SVCV-G 2E1	5 tubes	2015-15795
Sweden	Eva Blomkvist	National Veterinary Institute	30.09.2015	Eel tissue and supernantants for identification	7 tubes	2015-18128
Italy	Anna Toffan	OIE National Reference Laboratory for VET	07.10.2015	Rainbow trout, Supernatant for diagnostic confirmation and sequencing	2 tubes	2015-18464

Country	Name	Institute	Date of receipt	Material	Amount	Protocol No.
				Rainbow trout, Supernatant for diagnostic confirmation/identification	1 tubes	2015-18465
				Distinct IHNV viral strains for forwarding	3 tubes	2015-18466
Iceland	Sigridur Gudmundsdottir	Institute for Experimental Pathology	27.10.2015	Supernatant of EPC cell culture from Lumpfisk (kidney/heart) infected with unidentified virus for diagnostic testing.	4 tubes	2015-19852
Switzerland	Thomas Wahli	University of Bern	18.11.2015	Rainbow trout. Cell culture supernantant. IHNV isolates.	4 tubes	2015-21831
The Netherlands	Olga L.M. Haenen	Central Veterinary Institute (CVI) of Wageningen UR	26.11.2015	Carp, supernatant, controls for CEV PCR assay	2 tubes	2015-22608
Greece	Georgios Spiliopoulus	Andromeda Group	17.12.2015	Whole fish for virological and bacterial examination	15	2015-23618
Slovenia	Vlasta Jencic	University of Ljubljana, Veterinary Faculty.	23.12.2015	Rainbow trout, kidney tissue for BKD examination	30 tubes	2016-456

Annex 3. Laboratory visit at the National Reference Laboratory for Fish Diseases Institute for Experimental Pathology, KeldurUniversity of Iceland

Reykjavík-Keldur, Iceland
26th – 27th November 2015

Introduction

The National Reference Laboratory for Fish Diseases (NRL) in Iceland is located at the Institute for Experimental Pathology at Keldur, Keldnavegi 3 112 Reykjavik, Iceland. The Institute was visited from the 26th to the 27th of November 2015 by Niccolò Vendramin and Teena Vendel Klinge from the European Union Reference Laboratory for Fish Diseases (EURL). The program for the visit is shown in Annex 1 and a list of persons met by the EURL representative is listed in Annex 2. This report describes findings, comments and recommendations made by the delegation from the EURL. This report is sent to the NRL and the EU Commission.

Organisation

The Institute for Experimental Pathology operates according to a statute of the Icelandic parliament enacted in 1990. It is an academic establishment and is affiliated with the Faculty of Medicine University of Iceland, with a special governing board and an independent budget. The predominant aspects of the activities are basic and service research in veterinary medicine.
The expertice is on the following disciplines: prionology, virology, bacteriology, parasitology, pathology, immunology, biochemistry and molecular biology.
Both animal husbandry and general farming are conducted at the Institute in connection with production, services and research.

The Institute for Experimental Pathology is managed by **Director: professor** Sigurður Ingvarsson. The Institute for Experimental Pathology is divided into 4 departments/divisions: Office-Farming, Division for fish diseases, Division for Bacteriology Pathology and Parasitology, Department of Virology and molecular biology. An organization plan is shown in Annex 3.

The fish diseases laboratory at Keldur is appointed as national reference laboratory for:

- Fish diseases
- Mollusc disesases
- Crustaceans diseases

The Institute for Experimental Pathology employs a total of 46 persons

Buildings, Furnishing and Access
The Institute for Experimental Pathology is placed in three main buildings at Keldur in proximity of Reykjavík.

All access to the institute buildings is controlled through keys. There is free access between the buildings but entrances to the different laboratories are restricted through locks. Rooms for cell cultivation and PCR have controlled temperature in order to maintain the environment cooled (approx. 16 degrees Celsius in cell culture facility).

Meeting Organization
The meeting was divided into main topics.
On the 26th after the introduction to the laboratory the following topics were discussed:

- Introduction to the laboratory, main activities carried out, presentation of the staff involved in the area
- Laboratory procedures and Proficiency test results
- Visit of the facilities, description of working flows, quality insurance system, traceability system for diagnostic samples receive

On the 27th it was organized a field visit with two core objectives.

- Visit of a farm producing lumpfish for biological contrast to sea lice parasites. Fish are exported, alive, mainly to the Faroe Island. This visit was conducted together with veterinary officer responsible for aquaculture in Iceland Gísli Jónsson.
- Visit at research facility in Sandgerdi where in vivo trial in fish can be conducted.

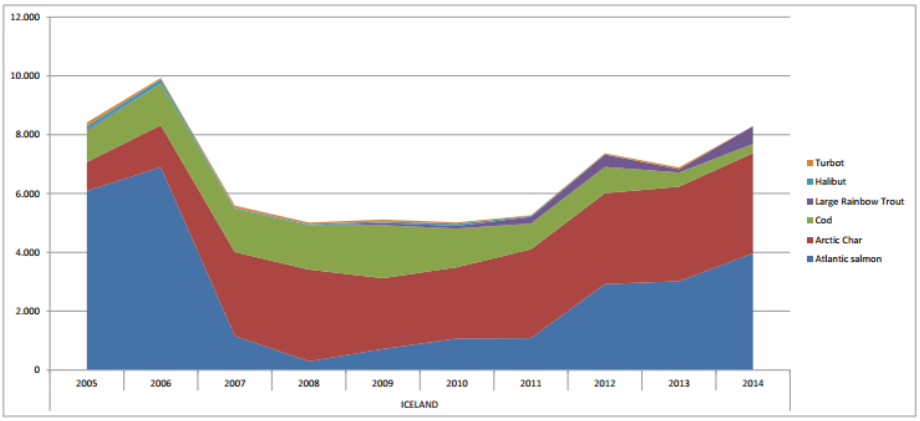
November 26th Opening of the meeting
The meeting lasted for one and half day, including the whole day on the 26th and the morning of the 27th.
The official opening took place on the 26th November at 9:00 where Árni Kristmundsson, head of the fish diseases laboratory provided a presentation of the activities run at the laboratory in Iceland (a handsout version of the presentation is add as Annex 6).
The meeting continued dialoguing on performances of Inter-laboratory Proficiency Tests (PT) and the results obtained during the PT2015.
After the coffee break a short tour of the institute where description of the institute and structure of the laboratory for fish pathology was given. The tour was guided by Sigríður Guðmundsdóttir Heiða Sigurðardóttir Edda Björk Ármannsdóttir

Aquaculture in Iceland and related surveillance and diagnostic activity for fish diseases

Iceland production (tons) 2005-2014

PRODUCTION (tons)		YEAR									
COUNTRY	SPECIES	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
ICELAND	Atlantic salmon	6.094	6.895	1.158	292	714	1.068	1.083	2.923	3.018	3.965
	Arctic Char	977	1.426	2.851	3.124	2.405	2.427	3.021	3.089	3.215	3.411
	Large Rainbow Trout	50	10	11	6	75	88	226	422	113	603
	Cod	1.050	1.412	1.467	1.502	1.805	1.317	877	893	482	310
	Turbot	115	47	70	51	68	46	20	28	58	0
	Halibut	129	141	31	39	49	72	33	13	0	0
ICELAND Total		8.415	9.931	5.588	5.014	5.116	5.018	5.260	7.368	6.886	8.289

SOURCE: Icelandic Aquaculture Association



As described by the figures provided by FEAP, aquaculture in Iceland is a rather developed activity and every year approximately 8300 Tons of fish were produced in 2014. The most important part of this production consist of Atlantic salmon (*Salmo salar*) 3,9 MTonns and Arctic charr (*Salvelinus alpinus*) 3,4 Mtonns. Cod is still farmed to minor extent, and small productions are relying on flatfish (turbot, sole). A new species which has gained a lot of interest in relation to control of lice in salmon net pens is cleaner fish, especially lumpsucker (*Cyclopterus lumpus*).

Pathogens and related diseases investigated at the fish disease laboratory

<u>Routine health surveillance- Viruses</u>

Viral routine health surveillance is performed either with cell culture or qRT-PCR based methods depending of the virus that are investigated.

Concerning IHNV, VHSV, IPNV, EHNV, sub-samples of brood fish from all fish farms and juvenile fish for export or according to special agreement (e.g. *Senegalese sole, cod, halibut, turbot*) are investigated with Cell culture using EPC and BF-2 Cell monolayer. The screening of VHSV, IHNV and IPNV using cell culture is accredited by SWEDAC (IST EN ISO/IEC 17025).

Regarding ISAV, SAV (PD), IPNV, PMCV (Piscine Myocardiopathic Virus) the methods focus on screening prior to export of salmon eggs. These pathogens are investigated with qRT-PCR. The Screening activity of ISAV, SAV (PD), IPNV, PMCV using RT qPCR is accredited also by SWEDAC (IST EN ISO/IEC 17025).

Routine health surveillance- Bacteria
Renibacterium salmoninarum, the aetiological agent of Bacterial kidney disease- BKD has a major importance in the activities carried out at the fish disease laboratory at Keldur, this is related mainly to the re-stocking activities conducted with salmon populations in the different rivers.
All wild female brood fish used for restocking of rivers are screened. Farmed fish: Sub-samples of female brood fish from all farms. Methods: ELISA, FAT, culture, PCR

Consider the overall activity performed by the lab., there is an annual turnover of 9.500 samples investigated .

The Fish Disease Laboratory

The Fish disease laboratory is an autonomous entity within the institute for experimental pathology and is responsible for diagnostics of diseases of aquatic animals from farms and open water. The division for fish diseases coordinates the NRL for fish-, crustacean- and mollusk- diseases. The NRL for fish diseases includes molecular based fish diagnostics while histopathological methods are the main diagnostic tools regarding diseases of crustaceans and molluscs.

Staff of the Fish Disease Laboratory
The Fish disease laboratory consists of a group four and a half persons fully allocated to the laboratory: Árni Kristmundsson in charge of the pathology laboratory and parasitic diseases, Sigríður Guðmundsdóttir in charge of virological analysis; Sigríður Hjartardóttir in charge of bacteriological analysis and Heiða Sigurðardóttir and Edda Björk Ármannsdóttir in charge of the molecular biology laboratory.
The laboratory get support from other employees at the institute partly allocated to fish diseases and employs students (MSc and Phd) to carry out research projects

The Fish Disease Laboratory
The laboratory is well equipped, and the space allocated to laboratory is appropriate, it positively impress how the work flow is organized, keeping space separation between different part of the laboratories (sample preparation, “dirty room” for inoculation of cells and virus identification and clean room for pathogen free cell preparation).

PCR based diagnostics
The laboratory for Fish diseases contains equipment for performing PCR based fish diagnostic analyses that is located in a laboratory in another building on the premises. Diagnostic PCRs and RT-PCRs are performed by Heiða Sigurðardóttir and Edda Björk Ármannsdóttir. A number of diagnostic qRT- PCR have been validated through SWEDAC including analysis for ISAV, SAV (PD), IPNV, PMCV.

Sample acceptance and reporting unit
The visit tour started in the room used for acceptance of samples, fish necropsy and ELISA for BKD.
Samples from fish farms are generally delivered directly to the fish disease laboratory, whereas rarely to the general acceptance. All samples, after acceptance registered and given a unique number which will allow to trace back samples and analysis when the report is issued to the authorities or fish farmer. The laboratory for fish diseases at institute for experimental pathology relies on LIMS system for tracing samples and analysis. The room for acceptance of samples is furnished with rather big cooler where samples are stored before analysis is started. When the results of the diagnostic analyses have been obtained a final report is made in LIMS. This answers

has to be signed by the leading researcher or the head of the laboratory, before it is send to the customer.

Cell culture facility

2 separate rooms are allocated to the cell culture facility. One room is allocated to maintenance and production of cell culture for diagnostic purposes, so to speak a “clean” cell culture facility. This room has 2 incubators and LAF bench. One room is allocated to inoculation of cell culture monolayer with supernatant obtained from diagnostic samples, this activity is conducted in a separate LAF bench, and cells incubated in a different incubator. The microscope to assess the presence of cytopathic effect – CPE is shared between the rooms, however flow separation is maintained timewise and disinfection is carried on in between. Interestingly high biosecurity measures are implemented for these rooms, including change of shoes and lab. Coat; finally room temperature is kept at +16 degrees Celsius.

PCR Facility

One room host the PCR facility, different cabinet are used for the different phase of the PCR process, one for RNA-DNA purification and one for Mastermix preparation. The room is equipped with 2 Q-PCR machines that are maintained under accreditation and where the analysis under accreditation quality insurance system are performed, furthermore another Q-PCR machine is used for research studies and used mainly by students. Interestingly high biosecurity measures are implemented for these rooms, including change of shoes and lab. Coat; finally room temperature is kept at +20 degrees Celsius.

Summary of suggestions

- Implement ELISA techniques for identification of viral isolates from BF-2 and EPC cell culture monolayers as it is considered a simpler technique to transfer and teach to the laboratory staff.
- Since Senegalese sole are imported and farmed in Iceland, Nodavirus should be one of the pathogens that the laboratory should be able to investigate. A diagnostic method either based on cell culture (SSN-1) or Real Time q PCR could be considered to be implemented.

November 27th Visit at cleaner fish facility and facility for conducting "in vivo" trial in Iceland

The morning of the last day was allocated to field trip.
The first facility visited was rearing Cleaner fish; a farm which was affected by the Ranavirus in early summer 2015. Subsequently all fish present at the farm at that time were discarded. The other farm, i.e. the one that experienced a VHSV outbreak was empty and all fish has been removed.
Interesting knowledge on needs for rearing cleaner fish were gained and opportunity to transfer live cleaner fish to the facilities of DTU VET were discussed.
The second point of the visit was the facility where the Icelandic team can carry out in vivo experiments located at Sandgerdi 50km from Reykjavik. A presentation of the team working in the facility and opportunity there to carry out infectious trial were described.

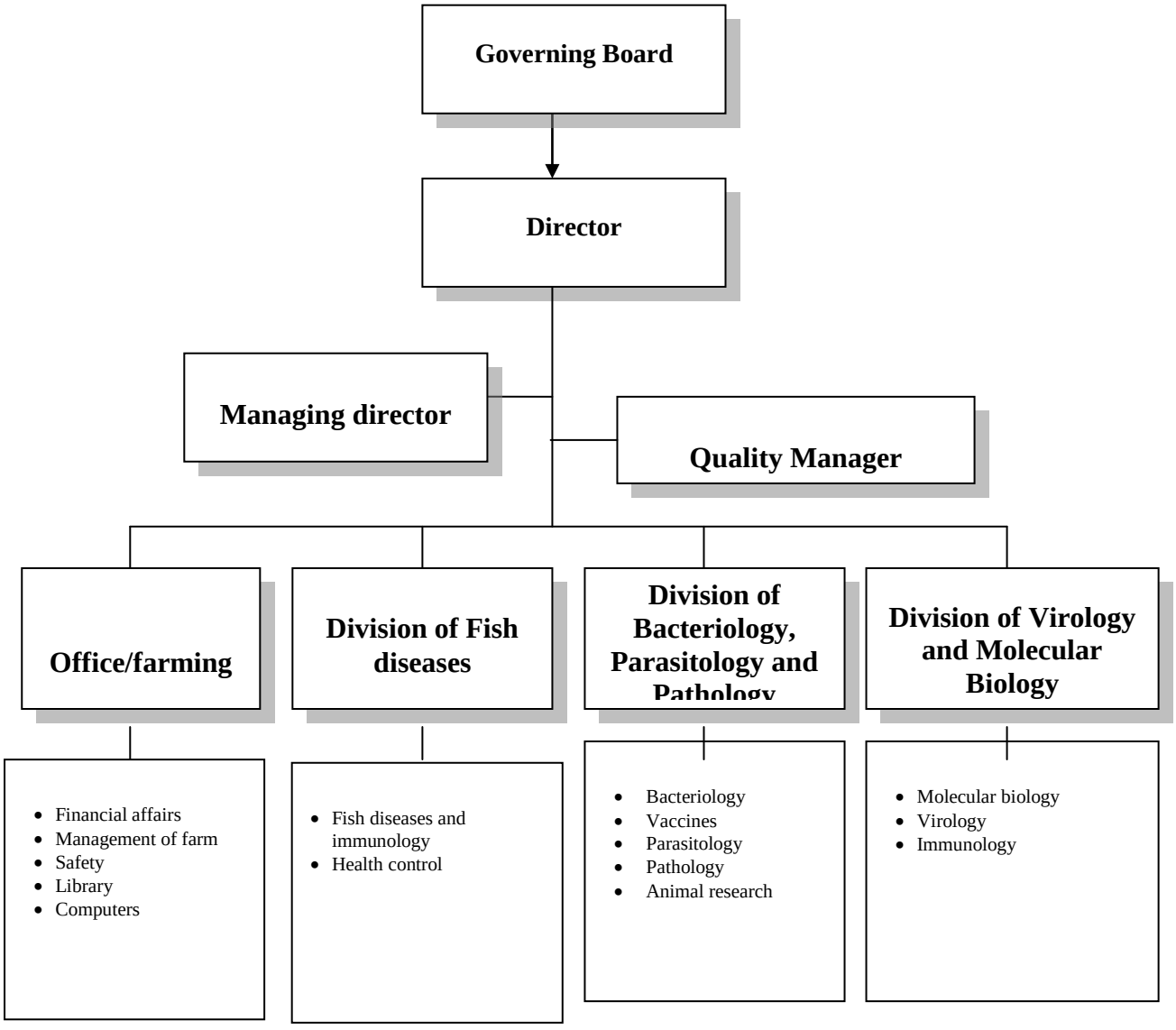
Final conclusion

The visit to the Icelandic laboratory was regarded as success. As demonstrated by high scores obtained in Proficiency Tests of the past years, diagnostic procedures for listed fish diseases are performed adequately. The visit has also been successful to boost the cooperative activities going on between Icelandic NRL and EURL under the perspective of monitoring and promptly reacting when VHSV cases come up

Annex 1 Organisational and Functional Structure

INSTITUTE FOR EXPERIMENTAL PATHOLOGY, UNIVERSITY OF ICELAND, KELDUR

ORGANISATIONAL CHART



Annex 2 Aquaculture in Iceland

Survey & Diagnosis of listed fish diseases in Europe 2013						
	Country / Region:	**ICELAND**				
1. General data, whole country or region (as of 31.12.2013)		**Answer**				
1.1 Number of fish farms within country/region, according to size of production (tonnes fish/year) (the sum has to be = the total number of farms in the country)						
	< 5 tonnes	12				
	5 - 100 tonnes	24				
	> 100 tonnes	15				
1.2 Number of fish farms within country/region, according to fish species (if more than one fish species is present in the farm, this farm has to be reported for every fish species)						
	Rainbow trout	10				
	Atlantic Salmon	13				
	Other salmonids	29				
	Carp	0				
	Eel	0				
	Flatfish	3				
	Sea bream / Sea bass	0				
	Other marine spp.	5				
	Other freshwater spp.	1				
1.3 According to Council Directive 2006/88, please indicate number of farms in your country/region placed in zones/compartments with the respective categories according to fish species:						
Category I Declared disease-free		**VHS**	**IHN**	**ISA**	**KHV**	
	according to susceptible species lists in Part II of Council directive 2006/88/EC	27	27	3		
Category II Subject to a surveillance programme		**VHS**	**IHN**	**ISA**	**KHV**	
	according to susceptible species lists in Part II of Council directive 2006/88/EC					
Category III Not known to be infected but not subject to surveillance programme for achieving disease free status		**VHS**	**IHN**	**ISA**	**KHV**	
	according to susceptible species lists in Part II of Council directive 2006/88/EC			10		
Category IV Known to be infected but subject to an eradication programme		**VHS**	**IHN**	**ISA**	**KHV**	
	according to susceptible species lists in Part II of Council directive 2006/88/EC					
Category V Known to be infected. Subject to minimum control measures		**VHS**	**IHN**	**ISA**	**KHV**	
	according to susceptible species lists in Part II of Council directive 2006/88/EC					
Do you have any comments on the categorisation of farms and zones in your country? Please notice that the questionnaire only asks for number of farms not zones. If possible we would appreciate to receive a map of your country indicating the categorisation according to the respective non-exotic diseases.						
1.4 Number of fish farms surveyed for other diseases						
Disease	**Programme based on: National / Regional -, Industry programmes or Research survey. Please specify e.g. National surveillance based on inspections (passive surveillance), IPN Brood stock testing, Industry financed or regional BKD program**	**Number of fish farms involved**				
SVC						
BKD	National BKD program running since 1985 (Industry financed)	15				
IPN	National program, mainly based on IPN brood stock testing, running since 1985 (Industry financed)	10				
Gyrodactylus salaris	National surveillance based on inspections (passive surveillance) and microscopical survey, in farms rearing wild salmon fingerlings released back to salmon rivers	5				
Other (like PD, SD, HSMI, CMS)	National surveillance based on health inspections, tissue samples and laboratory work mainly regarding PD/SAV, CMS and HSMI (Industry financed)	5				
1.5 Do you have targeted, active or passive surveillance for the exotic fish diseases in Directive 2006/88?						
Disease	**Targeted *(yes/no)***	**Active (yes/no)**		**Passive (yes/no)**		
EHN	No		No	Yes		

2. Epidemiological data, whole country or region (as of 31.12.2013)		Answer
2.1 Number of fish farms considered to be infected for the following diseases (NB: not necessarily officially declared infected)(if none write 0):		
	VHS	0
	IHN	0
	KHV	
	ISA	0
2.2 Number of fish farms with susceptible species that are considered to be free of the following diseases according to national surveillance (NB: not necessarily officially declared free):		
	VHS	27
	IHN	27
	KHV	
	ISA	13
2.3 Number of fish farms with susceptible species for which the infection status of the following diseases is unknown (total number of farms excluding farms considered to be free and considered to be infected. NB: not necessarily officially declared infected/free):		
	VHS	0
	IHN	0
	KHV	0
	ISA	0
2.4 Describe problems with fish diseases in your country other than VHS, IHN, KHV or ISA:		
	Fish species	*Please describe disease or symptoms*
2.5 Is there an increase or decrease in the number of fish farms infected with listed diseases compared to previous years? If yes please specify:		
	NO	
2.6 Is there a general increase or decrease in the severity of infections with listed diseases compared to previous years? If yes please specify:		
	NO	

3. Laboratory data, NRL and regional laboratories in 2013		Answer	
3.1 Number of fish samples (pools of tissue material) examined virologically (in cell cultures and by direct methods without cell cultivation) in NRL and regional laboratories, in total:			
		No. of samples tested by cell cultivation	**No of samples tested by PCR or other direct methods without cell cultivation**
	No. of samples		
3.2 Number of samples tested positive in NRL and regional laboratories in your country:			
Number of virus positive samples		**No. of samples tested positive by cell cultivation**	**No of samples tested positive by PCR or other direct methods without cell cultivation**
	VHSV		
	IHNV		
	ISAV		
	KHV		
	SVCV		
	IPNV		
	PDV/SDV		
	Nodavirus		
	Other:		

3.3 Other virus or pathogens identified (e.g. *A. invadans, G. salaris* , Piscine reovirus, Piscine myocarditis virus, *R. salmoninarum* , *Francisella* , *Piscirickettsia* etc.):

Fish species	*Virus or other pathogen*	*Number of fish farms/cases*

Data from S&D 2014