European Union Reference Laboratory for Fish Diseases

National Veterinary Institute, Technical University of Denmark, Copenhagen

Technical Report 2014

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

Content of Annexes

Annex 1: Reagents received at the EURL in 2014

Annex 2: Reagents supplied by the EURL in 2014

Annex 3: Report from mission to Croatia

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 and the Commission Implementing Decision of 17th January 2014 as regards a Union financial aid for the year 2014 to European Union reference laboratories (2014/27/EU).

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

Again this year an inter-laboratory proficiency test was distributed to the NRLs mainly within the EU but there were also participants from countries outside EU. For the fifth year, the proficiency test consisted of two tests, PT1 and PT2. PT1 was designed to primarily assess the ability of participating laboratories to identify VHSV, IHNV and EHNV + SVCV and IPNV. PT2 was developed in order to test the proficiency of participating laboratories to identify ISA and KHV. The proficiency test is covering all 5 listed exotic and non-exotic diseases. 41 National Reference Laboratories (NRLs) participated in the proficiency test. A report was submitted primo March 2015. Most laboratories performed well.

An important focus of the EURL is to update the standard operating procedures of the non-exotic and exotic listed diseases. Diagnostic manuals for VHS, IHN, ISA, KHV and EHN are available at the EURL web page. In order to update the sampling and diagnostic procedures for KHV the EURL invited 3 experts for a 2 day scientific meeting and passed the outcome to the Commission in order to recommend and finally adopt a Commission Decision on KHV along with the other non-exotic aquatic animal diseases. The Commission Decision on sampling and diagnostic procedures for the listed non-exotic diseases are expected to be adopted by the Commission primo 2015 and will be finally in force as soon as accepted.

Another important focus area was the development, implementation and validation of diagnostic tools for identification of the listed and emerging diseases and their accreditation. One outcome of these efforts was the implementation of a real time RT-PCR for detection of virusY, the putative causative agent of a new disease observed in Rainbow trout in Norway.

During 2014, resources were again used to collate data on surveillance, health categorisation, and diagnostics in EU; to identify and characterise selected virus isolates; to type, store and update a library of listed virus isolates; to develop, update and maintain the database containing information on fish pathogens (www.fishpathogens.eu); to supply reference materials to NRLs; to provide training courses in laboratory diagnosis; to produce anti-sera; to update the EURL webpage (www.eurl-fish.eu); and finally to attend international meetings and conferences.

In 2014 the fish diseases activities of DTU Veterinary were established in Copenhagen after the transfer from Aarhus in 2013. The number of colleagues within the group is now 5 academics and 4 technicians, but the EURL still collaborate with the fish diseases research group conducted by prof. Niels Lorenzen who, due to the transfer, jumped from DTU to Aarhus University.
The new placement also resulted in a close localization together with scientists conducting the function as NRL for mollusc diseases and who are internationally recognised researchers in fish bacteriology, as well as close distance to excellent scientists and research facilities in the Copenhagen area.

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, 31 March 2015

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 2014 – 31 December 2014

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 2014

Technical report

1. Coordination and training

1-1,1-2 Organise and prepare for the 18th Annual Workshop for the National Reference Laboratories for Fish Diseases (NRLs) and produce a report from the Annual Workshop

Organization of the 18th Annual Workshop

The 18th Annual Workshop of the National Reference Laboratories for Fish Diseases was held in Copenhagen, Denmark, 3-4 June 2014 at the Auditorium of DTU Veterinary Institute in Bülowsvej 27, 1870 Frederiksberg C.

A total of 53 participants from 32 countries attended over the two days period. There were five sessions with a total of 29 presentations, 2 of which were given by invited speakers, and a working group session.

The scientific program of the Annual Workshop was wide and covered many different topics of current interest. The meeting was opened with the traditional session "Update on important fish diseases in Europe and their control", where participants from the Member States had the opportunity to present new findings from their respective countries.

Initially, an overview of the disease situation and surveillance in Europe 2013 was provided on the basis of the results obtained from the Survey & Diagnosis questionnaire.

Then the fish disease situation in Norway was presented; a detailed report is available at: http://www.vetinst.no/eng/Publications/Fish-Health-Report.

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

This was followed by an update on VHS and IHN recent outbreaks that occurred in Croatia shortly after its entry in the EU.

The talks of the first session were completed by presenting data on surveillance of Atlantic salmon and Arctic Charr in Iceland.

The second half of the morning was allocated to a new activity, introduced for the first time during the Workshop. Each participant was asked to consider the relevant infectious diseases for the 2 most important fish species farmed in his country. After this first level of investigation, representatives of different macro-areas in Europe were grouped. The regions were Northern Europe, gathering the main Salmon producing countries, Eastern Europe focusing mainly on cyprinids and subsequently rainbow trout, Western Europe producing mainly rainbow trout and cyprinids and finally Southern Europe producing mainly the marine species European sea bass and gilthead sea bream and then rainbow trout. Experts had the possibility to discuss and describe the impact of each disease focusing on the 3 most important parameters. The first topic considered was the impact of a given disease on production, meaning the severity of losses in terms of mortalities, reduction of growth, etc. Then impact on the economy, indicating the cost of prevention (i.e. vaccination and biosecurity measures), treatment, reduced value of the product was considered. Finally consequences due to trade restrictions, national plans for control/eradication, suspension time after antibiotic treatment etc. Each group had to finalize its task by selecting and describing the most important diseases.

During the final part of the session a representative of each area described the agreed findings to the whole assembly.

The second session was dedicated to Emerging diseases.
Firstly, the Swedish representative presented an update on infectious disease status describing unsolved cases and the appearance of haemorrhagic smolt syndrome - HSS.
The second talk gave an interesting and detailed overview of CMS and HSMI, two important viral diseases that affect Atlantic Salmon.
In connection to this topic, the results of a screening for Piscine Reovirus (PRV) causing HSMI and Piscine Myo-Cardiopathy virus (PMCV) causing CMS conducted on wild salmon used for restocking purposes in Denmark was presented.
A talk describing how new farming practices and technologies affect the appearance and severity of infectious diseases was given focusing on Danish aquaculture production using recirculating facilities.
Finally the new virusY, a virus causing a not previously described disease in Rainbow trout in Norway was addressed.

The third session, on control and surveillance of relevant pathogens in the EU, started by a presentation on surveillance and diagnosis of koi herpesvirus disease (KHVD).
Then the output from the work of an expert group established by the EURL on surveillance and diagnostic methods for KHV in order to finalize a draft of the new Commission decision Part 2 on sampling and diagnostic procedures for KHV was presented.
This was followed by an introduction to the new Aquatic Animal Health Law. In May 2013 the European Commission launched a proposal for a new EU Animal Health Regulation, and the work is currently in the process of being adopted by the legislative bodies of the Union. The Regulation aligns with the Lisbon Treaty and lay down horizontal principles and rules for transmissible animal diseases in kept and wild animals and their products.
Finally the health categorization of fish farms in Europe in 2013 based on answers from the questionnaire on “survey and diagnosis” that is delivered every year to all national reference laboratories was presented.
The second and last day was opened by the session on scientific research update; this fourth session faced several different topics covering molecular characterization of pathogens, a present and future core topic for all the laboratories involved in fish disease diagnosis, and vaccination as a strategy to prevent infectious diseases.
The session started with a presentation on Maldi-tof, a new diagnostic tool able to identify microbial pathogens; benefits and challenges with this new technology were described. Then a new tool for evaluating antimicrobial resistance in fish pathogens was described.
The session continued with an update on TARGETFISH, an FP7 granted project that focus on development of improved and targeted vaccination strategy for important farmed fish species in European Aquaculture.
This was followed by a presentation showing results of MOLTRAQ, an EMIDA ERA-NET funded project that focus on the molecular tracing of viral pathogens from aquatic animals.

The next presentation described findings of proliferative kidney disease (PKD) infection in wild trout in Denmark, focusing on the relation between wild and farmed species in the epidemiology of this important disease.
The selection of new candidate fish species in aquaculture has to consider health management, this topic was addressed by an interesting talk evaluating sensitivity of Redfin Perch and Marble Trout to VHS and IHN infection under experimental conditions.
An update on CEV, a virus appearing in carp in Europe was provided, stressing the need for the development of reliable diagnostic techniques.
As reliable validated techniques are beneficial for the whole Europe, the validation testing of serum-neutralisation allowing the detection of specific antibodies against Koi Herpes virus was given.
As a follow up on presentations from 2013, describing a VHS outbreak in wrasse used as cleaner fish in the Shetland Islands, the output of experimental trials and pathology of this disease in this species was provided.
Finally this session was concluded by presenting the findings of suspicion of VHS in Iran.

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

The final report of the 18th 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 2013

The Questionnaire on Surveillance and Diagnosis (S&D) which is collated annually is the only comprehensive overview of the disease situation in aquaculture in Europe. The information has been made available on the EURL web site (www.eurl-fish.eu), where all raw data can be obtained. The S&D have evolved and changed a bit over the years, for 2013 it comprise 3 parts:

1. General data on aquaculture fish production, type, and size, health categorization of fish farms according to Council Directive 2006/88/EC, and information on national surveillance programmes.
2. Epidemiological data on the disease situation in each Member State with focus on the listed diseases but also including other diseases of interest.
3. Laboratory data from the NRLs and other laboratories, including the numbers of samples examined, and diagnoses of fish diseases made.

The data on the European aquaculture production were obtained from the FIGIS database. This database does not include information on the number and size of fish farms, and therefore these data had to be obtained directly in the questionnaire. In FIGIS only data from back to 2012 is available. The production has increased quite significantly from 2011 to 2012. The increase

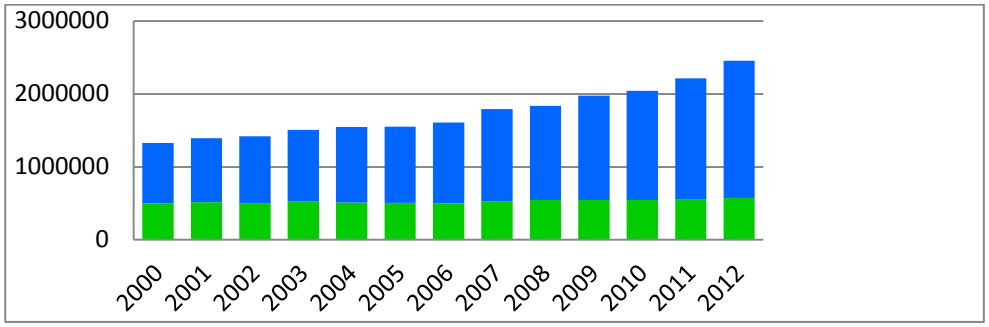
primarily account for the Atlantic salmon production, especially in Norway. With a rise from 0.68 mill t A. salmon in 2001 to 1,36 mill t in 2011 and 1.5 mill ton in 2012, Europe is following the global development towards increased aquaculture production (Figure 1). The rainbow trout production has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

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

Many countries have surveillance programmes for SVC (21 of 35 participating countries), BKD (17 of 35 countries), IPN (18 of 35 countries) and Gyrodactylus salaris (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, and Amoebic gill disease in the salmon production, but in 2012 in addition several countries reported findings 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 2012 (http://www.fao.org/figis)

A summary of the results for 2013 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

The EURL Fish is yearly offering 2 training courses in diagnostic techniques for identification of listed fish diseases.
The training courses took place at DTU National Veterinary Institute in Copenhagen, Denmark, from September the 8th to the 17th, 2014. 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 8th to 12th September 2014. The second course was entitled “Real-time PCR for diagnostics and surveillance of Fish Diseases” and took place 15th to 17th September 2014 with 13 participants. 3 persons 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. Staff of the EURL provided this training, together with teachers from the Danish Veterinary and Food Administration and CVI, The Netherlands. Also, knowledge-sharing and discussions between participants and teachers were important parts of the courses.

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 Niccolò Vendramin and presentations on Danish surveillance plan 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 and the morning was concluded with the introduction to the use of Real-Time PCR protocol for demonstrating freedom of listed fish diseases in Europe by Dr. Morten Bruun. 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 Lene Gertman were assigned, while for the real-time PCR part, Morten Sichlau Bruun, Troels Secher Rundqvist and Anemone Ojala 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.

Real-time PCR for diagnostics and surveillance of Fish Diseases.
The 3-days course in molecular techniques was devoted to lectures on relevant topics, as well as theoretical exercises.

Day 1 was dedicated to the basic PCR principles, sample preparation and validation of new assays. Lectures on the history and basic principles of PCR, and how to set up a lab and an assay to prevent contamination was given by Marc Engelsma and lectures on sample preparation, transport and storage and validation of new assays was given by Susie Sommer Mikkelsen, all with a focus on fish diagnostics. Furthermore, there was a discussion about the application of the MIQE guidelines. The day ended with theoretical exercises covering the areas taught earlier in the day. The participants were divided into three groups and given their own rooms to encourage discussion between them. Furthermore, both teachers moved between the groups to help answer questions and provide basis for more discussion.

Day 2 was primarily about the different PCR chemistries, primers and probes

and controls. Lectures on the different PCR chemistries and primer and probe design together with a live demonstration on how to create new primers was given by Susie Sommer Mikkelsen, while Marc Engelsma gave a lecture on the appropriate controls and standards for real-time PCR assays for diagnostic purposes. After the last lecture the participants were provided with theoretical exercises and encouraged to try and design their own primers on their laptops. Furthermore, both teachers moved between the groups to help and to encourage discussion.

The first part of day 3 was dedicated to lectures on thresholds, cut-off values and analysis and interpretation of results, provided by Marc Engelsma, exemplified using actual diagnostic cases. After the coffee-break, participants were invited to visit the facility for fish experiments at the Institute together with Torsten Snogdal Boutrup. 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, but participants generally would like more theoretical exercises to learn how to implement the knowledge gained from the lectures.

Through lectures, exercises, discussions, and knowledge exchange between participants, the knowledge on real-time PCR, 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.

Participant List

Name	Country	Course 1	Course 2
Anna Maria Eriksson-Kallion	Finland		X
Bartolomeo Gorgoglione	Austria	X	
Charlotte Axén	Sweden	X	
Christel Männistu	Estonia	X	
Christine Dubreuil	France		X
Daina Čavare	Latvia	X	
Dante Mateo	Canada	X	X
Dragan Brnić	Croatia		X
Eva Borzym	Poland		X
Haakon Hansen	Norway		X
Jacqueline Savage	UK	X	
Julia Jurovcikova	Slovakia		X
Laura Hawley	Canada		X
Lénaïg Louboutin	France	X	X
Lone Madsen	Denmark		X

Nadav Davidovich	Israel	X	
Nastaran Shahbzian	Iran	X	X
Simona Pileviciene	Lithuania		X
Stamatina Arfara	Greece	X	
Valentina Panzarin	Italy		X

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

Master and PhD students:
No master or phd students were affiliated to the EURL Fish in 2014

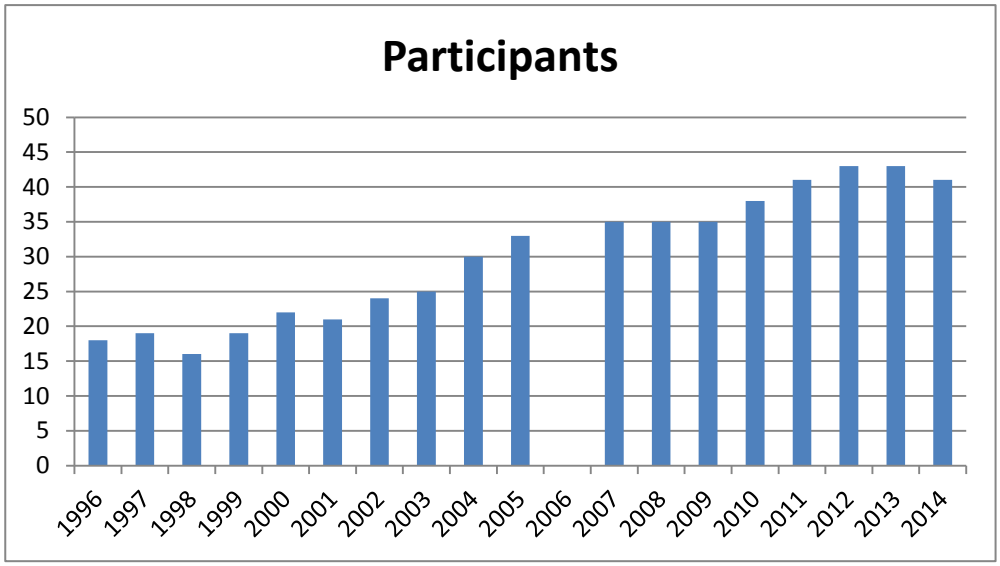
OIE collaboration:
N.J. Olesen is designated expert of the OIE reference laboratory for VHS, and in this function had consultancies and requests worldwide concerning VHS. The OIE chapter on VHS and related diseases in the diagnostic manual for aquatic animal diseases is revised and updated by the OIE reference laboratory for VHS.

2. Proficiency tests

2-1 Prepare the Annual Inter-laboratory Proficiency Tests year 2014 for the NRLs. The test 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 2014

Since 1996, eighteen inter-laboratory proficiency tests (PTs) have been organised by the EURL. The number of participants has increased from 18 to 41. 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.



PT1 was designed as the proficiency tests provided by the EURL in previous years to primarily assess the identification of viral haemorrhagic septicaemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV), epizootic haematopoietic necrosis virus (EHNV), spring viraemia of carp virus (SVCV), and infectious

pancreatic necrosis virus (IPNV) by cell culture based methods. PT2 was structured with the aim of assessing the ability of participating laboratories to identify by biomolecular methods (PCR based) the fish pathogens: infectious salmon anaemia virus (ISAV), and Cyprinid herpesvirus 3 (CyHV-3) (otherwise known as *koi herpes virus* - KHV) while *Aphanomyces invadans*, the causative agent of epizootic ulcerative syndrome (EUS), was removed after delisting the pathogen from the legislation. The number of National Reference Laboratories (NRLs) participating in PT1 and PT2 was 41.
The tests were sent from the EURL in the beginning of October 2014. Both PT1 and PT2 are accredited by DANAK under registration number 515 for proficiency testing according to the quality assurance standard DS/EN ISO/IEC 17043

PT1 consisted of five coded ampoules (I-V). These ampoules contained IPNV, EHNV, SVCV, IHNV and VHSV, respectively. In addition the participants were asked to quantify the viruses in PT1 by titration in order to assess the susceptibility of their fish cell lines for virus infection. Participants were encouraged to use their normal standard laboratory procedures. However, the identification should be performed according to the procedures laid down in Commission Decision 2001/183/EC using fish cell cultures followed by e.g. ELISA, PCR, immunofluorescence (IFAT) or neutralisation test.
If ranavirus was present in any of the ampoules, it was mandatory to perform sequence analysis or a restriction endonuclease analysis (REA) of the isolate in order to determine if the isolate was EHNV or another ranavirus and it was recommended to follow the procedures described in Chapter 2.3.1 in the OIE Manual of Diagnostic Tests for Aquatic Animals 2014. Laboratories were encouraged to identify VHSV and IHNV isolates as far as possible by means of genotyping in addition to the standard methods. It was recommended to use the genotype notification described in Einer-Jensen et *al*. (2004) for VHSV and in Kurath et *al*. (2003) for IHNV. Laboratories were encouraged to submit all sequencing results used for genotyping the isolates.
Five ampoules with lyophilised cell culture supernatant were delivered to all NRLs in the EU Member States, including Denmark, and likewise to the NRLs in Australia, Bosnia and Herzegovina, Canada, Croatia, Faroe Islands, Iceland, Japan, New Zealand, Norway, Serbia, Switzerland, Turkey, and 2 from P.R. China and South Korea and USA. A parcel shipped to one laboratory in USA was never delivered due to customer restriction.
The Belgian NRL covers both Belgium and Luxembourg and the Italian NRL covers Italy, Cyprus and Malta for identification of all listed diseases. Figure 3 shows the worldwide distribution of the participating NRLs.

PT2 consisted of four coded ampoules (VI-IX). The ampoules contained 1 ampoule with ISAV and 2 with KHV and furthermore 1 blank ampoule.
Participants were expected to use their normal PCR or real-time PCR methods for detection of the pathogens. It was not mandatory to grow KHV and ISAV on cell cultures in this test, but the viruses were not inactivated and, thus, it might have been possible to replicate them in cell cultures.

NRL´s receiving the EURL PTs in 2014

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

Outcome of the Inter-laboratory Proficiency Tests 2014

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

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

Concluding remarks PT1:

In the report on the proficiency tests, all viral titres submitted by participants for each cell line and ampoule, respectively were compared to each other. In this way, the titres obtained by each laboratory were plotted in relation to the combined submitted data set and each participating laboratory were able to compare the sensitivity of its cell lines to the sensitivity of those used by the other participants. We recommended all participants to evaluate the sensitivity of the cells used in their laboratory in relation to the diagnostic purpose. This year it was remarked that a problem with the batch of ampoules containing IPNV Ab appeared. This has been taken into consideration in the process of giving score to participants. This year variation between virus titres obtained in the various laboratories was more pronounced than usually with up to 6 log differences between highest and lowest titres. It might reflect variation in the stability of the virus in the respective batches. Special precautions will therefore be taken in the following PT´s to ensure uniformity of the amount of viable viruses in the ampoules.

Concluding remarks PT2:

Considering that this was the fifth time that the EURL provided a proficiency test on ISAV and KHV identification, we considered that most participants obtained very good results. All 39 laboratories testing for KHV identified the virus in ampoule VI and VIII! Out of 40 laboratories 39 laboratories identified Not *KHV or* ISAV in ampoule VII. With only one false positive this is much less than observed in the PTs from previous years. All 40 laboratories testing for ISAV identified the virus in ampoule IX. Thereby very significant improvements in the proficiency of

identifying and typing these pathogens has been observed during these past 5 years, especially in relation to the sensitivity, as this year the viral content in the ampoule was low. After the European Commission in autumn 2012 de-listed Epizootic Ulcerative Syndrome cause by *Aphanomyces invadans* it has been agreed not to include this pathogen anymore in the PT for fish diseases.
It is an appreciated matter of fact that many laboratories are putting efforts in performing genetic characterization of the isolates through sequence analysis as it is important that laboratories can discriminate between isolates as e.g. pathogenic ISAV strains with deletions in the HPR region and HPR0 strains.
Of the 24 laboratories sequencing the ISAV virus all found that the isolate was with deletion in segment 6 and thus not belonging to HPR0. Some of the participants also noticed that this year the HPR13 isolate from the Faroe Islands was used instead of the Glesvaer isolate that we have included in all the former PT´s.

The results given in this report will be further presented and discussed at the 19th Annual Workshop of National Reference Laboratories for Fish Diseases to be held 27-28 May 2015 in Copenhagen, Denmark.
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.

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

3. Reagents and products

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

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 2014 are listed in Annex 3.

Further details of the supplied materials are listed in Annex 1

3-2. Production of antisera against selected isolates when necessary

Production of antisera

In 2014 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, IHNV, KHV, 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 2014.

Further details of the received materials are listed in Annex 2

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

Library of tissue material from fish infected with listed pathogens

Our repository of positive naturally infected tissue from VHSV, IHNV and IPNV infected fish, has been maintained, as well as organ material from negative controls.

pathogens

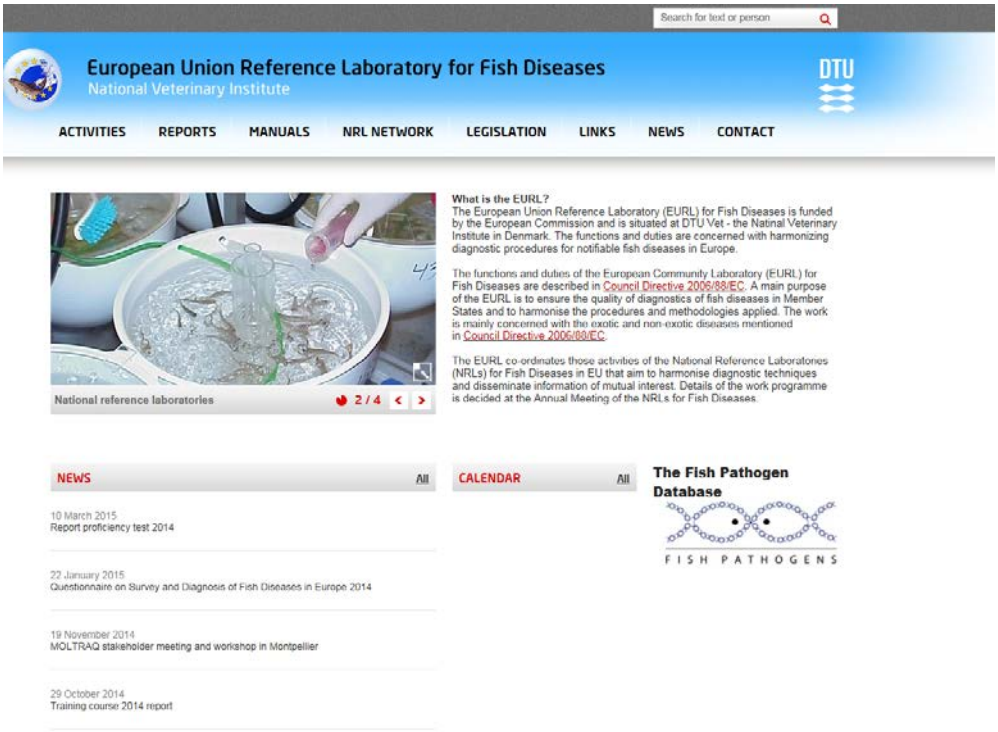
Tissue material from Atlantic salmon and rainbow trout infected with piscine reovirus (PRV) and rainbow trout infected with virusY have been stored as well.

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

According to the Council Directive 2006/88/EC additional legislation should be implemented describing sampling and diagnostic procedures for the diseases listed in Annex IV Part 2 of the Directive.The Sampling plans and the diagnostic methods for the detection and confirmation of VHS and IHN are laid down in the Commission Decision 2001/183/EC and for ISA in Commission Decision 2003/466/EC. But as KHV was only included as a non-exotic disease at the implementation of the Council Directive no earlier decisions were made for this disease. A preliminary version was given at the web page of the EURL Fish, this version was based on research and meeting activities reported in "Report of the workshop "KHV PCR diagnosis and surveillance" 12-13 November 2009, Central Veterinary Institute, Lelystad,

The Netherlands" and made by a KHV expert working group under the EPIZONE network. But as significant new knowledge based on new research appeared in the recent years, the EURL asked the Commission the permission to organize an expert meeting in order to settle an agreement on common new recommendations for implementation in a Commission Decision.
The 2 day meeting was held at the premises of the EURL at Frederiksberg, Denmark and 3 of the top experts in the field of KHV from Germany, Netherlands and UK, respectively, participated. The meeting was busy and held in a good atmosphere and at the end we finalized both versions of the surveillance and sampling methods and of the diagnostic methods. Significant changes from the former versions were accepted and recommended for inclusion in the final decision. Among changes were

- The splitting of sampling and diagnostic tests for diagnostic and surveillance purposes, respectively.
- Inclusion of real-time PCR as the method of choice for surveillance.
- Specification on how to define a CyHV-3 strain.

The final meeting report is available on the EURL web site
http://www.eurl-fish.eu/Diagnostic_Manuals

The most updated versions of the diagnostic manuals for VHS, IHN, ISA, KHV, EHN disease and EUS have all been prepared and are available from the EURL web page.

4-3 Fishreflabnet: Maintain and further develop the interactive network with the NRLs, Fishreflabnet , in order to promote a more proactive data sharing and communication with and between reference laboratories in member states.

Fishreflabnet:

A new 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:

VHSV: The full length G-genes of a significant number of Danish VHSV isolates (>80) were sequenced by the EURL and aligned for molecular tracing of pastVHS outbreaks in Denmark.

Identification and characterisation of selected virus isolates:

Member States and countries outside EU		
Material received	**Laboratories**	**Units**

Diagnostic material for virology	6	56 samples
Diagnostic material for PCR	8	67 samples
Diagnostic material for bacteriology	1	1 sample
PCR control material	1	2 sample
Serum	1	88 samples
Other material	0	0 samples

In 2014 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.

Further details are listed in Annex 5

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:

Veterinary Institute, Croatia (Snjezana Zrncic): Sea bass, brain tissue for virus examination suspicion of Noda virus infection. Noda virus not detected (DTU-VET 14-1734).

Veterinary Institute, Croatia (Snjezana Zrncic): Sea Bass. Tissue fixed and embedded for histopathology. Rickettsia spp. detected by IHC. (DTU-VET 14-2578).

Veterinary Institute, Croatia (Snjezana Zrncic): Rainbow trout, supernatant from EPC and BF-2 cells for virus examination and sequencing.
For cases 14-1731 and 14-1732 the isolates were confirmed to be Infectious haematopoietic Necrosis by ELISA and real-time PCR. The sequences place these two isolates in genogroup E.
For case 14-1733, it was demonstrated to be a co-infection case where it was possible to isolate both Viral Haemorragic Septicemia Virus (VHSV) and Infectious Haematopoietic Necrosis Virus (IHNV). The VHSV was confirmed by ELISA and real-time PCR, and was subsequently sequenced. Sequencing placed the isolate in genotype Ia, subtype 2, and it grouped into subclade Pol II. (DTU-VET 14-1733). The IHNV strain was demonstrated only by PCR and subsequently sequenced. Sequencing placed the isolate in the genogroup E.

National Veterinary Institute, Sweden (Eva Blomqvist): Salmon organ material collected during mortality outbreak were tested for Infectious Salmon Anemia Virus and Salmonid Alphavirus in order to rule out these pathogens. Both viruses not detected (DTU-VET 2014-50-40)

INRA, France (Pierre Boudinot): 88 sera from experimental vaccinated fish with different vaccines were tested in ELISA for detection of specific antibodies against VHSV. Results obtained were consistent with expectations, meaning that fish vaccinated against VHSV displayed specific antibody response. On the contrary fish mock-vaccinated or vaccinated with IPNV did not show any specific immune response.

Veterinary Institute, Ljubljana (Vlasta Jencic) Samples delivered for suspicion of BKD (DTU-VET 2014-50 -2). Samples tested suspected by direct antigen ELISA and positive with PCR

Inland Water Aquaculture Institute, Iran (Mohaddes Ghasemi) IPNV isolated on cell culture and identified by ELISA and IPNV specific viral RNA

detected by RT-PCR in one pool of organ from rainbow trout (sample 1) and BF-2 supernatant inoculated with rainbow trout fry (sample 10). VHSV, IHNV, KHV, SVCV or Noda were not detected in any of the samples.

Institute of Veterinary Medicine of Serbia (Vladimir Radosavljevic) Rainbow trout, spleen, heart, kidney for SAV detection and sequencing; samples tested positive by RT-qPCR and it was possible to isolate the virus from all three organs. (DTU-VET 14-2965)
Supernatant from cells inoculated with organs from Ameiurus (Ictalurus) nebulosus tested positive with Ranavirus specific PCR, sequencing confirmed that the isolate is European Sheatfish Virus. (DTU-VET 14-2965)

Institute of Veterinary Medicine of Serbia (Vladimir Radosavljevic) Carp, gill and kidney tissue from suspected KHVD outbreak were inoculated on CCB cells and tested directly with PCR. Specific PCR product consistent with KHV was sequenced and the presence of the virus DNA confirmed (DTU-VET 14-9691)

Islamic Republic of Iran Veterinary Organization (Mehdi Khalaj)
5 cell culture supernatant from cells inoculated with Rainbow trout organs and 7 samples consisting in organs material from rainbow trout were tested for virological examination and PCR. One supernatant (sample 12) tested positive both on cell culture and RT-PCR for IPNV

Norwegian Veterinary Insitute, Oslo (Monika Hjortaas): positive control material for VirusY was received and tested to implement RT-qPCR for diagnostic purpose and start infection trial to assess the pathogenicity of this new pathonge for European aquaculture (see paragraph 4.7) (DTU-VET 14-16130)

Veterinary Institute, Croatia (Snjezana Zrncic): Sea bass tissue from disease outbreak. Nodavirus infection ruled out by virological examination, histological lesions observed in the brain. Isolation of *vibrio ordalii* from 2 samples. (DTU-VET 14-15924)

National Veterinary Institute, Sweden (Charlotte Axen): Rainbow trout, Supernatant and organs from Rainbow trout for SAV PCR. Samples tested negative (DTU-VET 14-2783)

Norwegian Life Science University (Øystein Evensen) Purified RNA from cell culture with IPN, PCR analysis for VHSV, IHNV, SVCV. All pathogens were ruled out. (DTU-VET 14-538)

Norwegian Life Science University (Øystein Evensen) Purified RNA from cell culture with IPN, PCR analysis for VHSV, IHNV, SVCV. All pathogens were ruled out. (DTU-VET 14-1143)

National Veterinary Institute, Uppsala (Charlotte Axen) organs from various fish species found moribund on the coast of Sweden were received for pathogens investigation. A virus was isolated on cell culture and tentatively characterized. Various IFAT provide indications that the virus belong to the vesiculoviruses in the rhabdovirus family. (DTU-VET 14-3006)

4-5 Update and expand

The pathogen database www.fishpathogens.eu
The new design of fishpathogens.eu has gone live. Furthermore the new betanoda

www.fishpathogens.eu with more pathogens.

database is now up and running with 59 isolate reports and 108 sequence reports. The sequence matcher function has been enhanced with color coding for each genotype and works in conjunction with all databases in fishpathogens.eu.

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 accepted for publication in Journal of Fish Diseases.

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

One paper from the EURL on molecular epidemiology was published in 2014: *Britt Bang Jensen, Annette Kjær Ersbøll, Henrik Korsholm, Helle Frank Skall and Niels Jørgen Olesen* (**2014**) Spatio-temporal risk factors for Viral Haemorrhagic Septicaemia (VHS) in Danish aquaculture. Diseases of Aquatic Organisms 109: 87–97

A fundament for studying molecular epidemiology is the access to significant amounts of reliable data on virus isolates and corresponding sequences data, and to connect these with classical epidemiological data such as prevalence, GIS, sampling strategies and number of positive samples in relation to negative samples. Part of this information is accessible in our database www.fishpathogens.eu and efforts have primarily been devoted to expand and refine this database in order to use the most updated tools for conducting the studies. A close collaboration between partners in Europe on the topic has been established through the "MOLTRAQ" FP7 project. The collaboration will result in the inclusion of VHSV and IHNV isolate and sequence information from more than 10 European countries which will

become freely available to all researchers.

4-7 Real-time PCR: Assessment and standardisation of real-time PCR tests for the diagnosis, identification and typing of the listed non-exotic fish diseases.

Assessment and standardisation of real-time PCR tests

A real-time PCR was assessed and finally tested in 2014 for the detection of the emerging disease PRV (Piscine Reovirus).

A new disease was described in freshwater rainbow trout in Norway in 2013 and a new virus, the so-called virusY was detected in diseased fish, but has not yet been confirmed as the causal agent of the disease. After negotiation with the Veterinary Institute in Norway a TA has been signed and a collaborative project initiated in order to assess if the new disease is emerging and a threat for European rainbow trout farming or not. The virus is a member of the orthoreovirus family. A real-time RT-PCR has been implemented and infection trials planned for 2015.

A collaborative project with Aquaculture Institute, Santiago de Compostela University, Spain resulted in a paper: “A novel multiplex RT-qPCR method based on dual-labeled probes suitable for typing all known genotypes of viral haemorrhagic septicaemia virus" which was accepted for publication in Journal of Fish Diseases.

4-8 Emerging diseases: In collaboration with specialised experts WW to review selected emerging fish diseases in Europe and assess their potential listing as exotic or non-exotic diseases

Emerging diseases:

PRV has recently been associated with heart and skeletal muscle inflammation (HSMI), a disease that has led to large mortalities in the Atlantic Salmon farming industry. The affected fish show lesions in the heart and the mortality rate may reach 20%. Therefore it was decided to implement a real-time RT-PCR in the lab to be able to detect PRV as well as conduct a study on the prevalence of PRV in the wild salmon population. The assay was developed using an adapted protocol by Palacios et al. (2010). During testing of wild salmon spawners from Danish river systems, several positive fish were found (11%). Eggs from infected spawners were divided into 2 groups. Eggs in the first group were disinfected while the eggs in the second group were not disinfected. After hatching in our premises, all fry tested positive both in the disinfected and the non-disinfected groups. Also rainbow trout fry that were co-habited with the infected Atlantic salmon progeny showed positive Ct values but seemed to free them self for virus after a couple of weeks.
The PRV isolates were also sequenced using a protocol adapted from Palacios et al (2010) and it was shown that there seems to be no specific geographic grouping of the isolates which corresponds to the long-distance nature of the salmon. This is in concordance with studies done in Norway.

A new disease in rainbow trout in Norway and virusY, see above.

In May 2014 mass mortalities in eelpout (*Zoarces viviparous*) started to occur along the Swedish south-east coast. A virus was isolated by cell culture, but complement ELISA for epizootic piscine viruses (VHSV, IHNV, IPNV, SVCV) were negative. Further investigations indicated that a rhabdovirus was present; however repeated tests for piscine rhabdoviruses (VHSV, IHNV, SVCV), Perch rhabdovirus and Hirame rhabdovirus were negative or inconclusive. By Illumina sequencing conducted at SVA, Sweden two sequences were identified showing 70% similarity to mandarin fish (Siniperca chuatsi) rhabdovirus, identifying it as a potential new rhabdovirus. The EURL is involved in the characterization of the new virus and assessing its putative significance as an emerging disease.

5. Missions

5-1 Missions: Organizing missions to relevant laboratories. Missions will focus on NRLs where on-site communication would be beneficial. As collaboration with NRLs in 3rd countries from where EU is importing large amount of fish

Missions to relevant laboratories

In 2014 two missions were conducted.

One in connection with the 3rd Global Conference of OIE Reference Centres - Incheon (Seoul), Korea (Rep. of), held 14–16 October 2014 where 2 day visits were made by prof N.J. Olesen, director of the EURL to the National Fishery Products Quality Management Service (NFQS), Seoul, in order to assess future collaborative studies. The laboratory was after a FVO inspection urged to collaborate with the EURL and is now participating in the inter-laboratory proficiency tests.

The National Reference Laboratory for Fish Diseases (NRL) in Croatia is located at the Croatian Veterinary Institute (CVI). The Institute was visited from the 17th to the 18th of December 2014 by Niccolò Vendramin, coordinator of the EURL. A program for the visit was set up and a number of persons from the Croatian 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 and conferences attended.

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 2014:

Participation at international conferences and meetings

MOLTRAQ-workshop Berlin 19th-23rd of May
PARTICIPANTS: Niels Jørgen Olesen, Morten Sichlau Bruun, Susie Sommer Mikkelsen

18th Annual Workshop of the National Reference Laboratories for Fish Disease. Copenhagen, 3-4 June, 2014.
PARTICIPANTS: Niels Jørgen Olesen, Torsten Snogdal Boutrup, Morten Sichlau Bruun, Susie Sommer Mikkelsen, Niccoló Vendramin

DAFINET Workshop: Fish Models in Research a 3 day Workshop and Ph.D. course, University of Copenhagen, Denmark, November 11th, 12th and 13th, 2014
PARTICIPANTS: Niels Jørgen Olesen, Torsten Snogdal Boutrup, Morten Sichlau Bruun, Niccoló Vendramin

7th International Conference of the American Aquatic Animal Health Society, Portland, Oregon, 31.08-05.09.2015
PARTICIPANT: Niels Jørgen Olesen

Third Global Conference of OIE Reference Centres: Challenges and expectations for the future, Seoul, Korea (Rep. of), 14–16 October 2014
PARTICIPANT: Niels Jørgen Olesen

9th International Symposium on Viruses of the Lower Vertebrates, Malaga, Spain, October 2014

PARTICIPANTS: Niels Jørgen Olesen, Susie Sommer Mikkelsen, Niccoló Vendramin

TargetFish FP7 2nd progess meeting, 22-25 September 2014, Barcelona, Spain
PARTICIPANT: Niels Jørgen Olesen

Presentations and posters

Presentations and posters

18th Annual Workshop of the National Reference laboratories for Fish Diseases,

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. Health Categorization of zones and compartments in Europe. *Niels Jørgen Olesen*
4. Molecular Tracing of Aquatic Viruses - MOLTRAQ. *Mikkelsen, Susie Sommer; Schuetze, H.; Korsholm, H.; Jensen, B. B.; Bruun, Morten Sichlau; Olesen, Niels Jørgen.*
5. Comparative analysis of sequences from PT 2013. *Mikkelsen, Susie Sommer*
6. PMCV and PRV occurrence in wild and farmed fish in Denmark. *Mikkelsen, S. S., Arnö, J. & Bruun, M. S.*
7. EURL Training courses. Topics and organization for courses 2014 *Niccolò Vendramin Susie Sommer Mikkelsen*
8. Results of the proficiency test, PT1 and PT2, 2013 *Niccolò Vendramin*
9. Koi herpesvirus disease (KHVD) surveillance and diagnosis. *Way, K.; Bergmann, S. M.; Engelsma, Marc; Mikkelsen, Susie Sommer; Vendramin, Niccolò; Olesen, Niels Jørgen.*

7th ISAAH, Portland, US:

10. Control and eradication of viral hemorrhagic septicemia in Danish aquaculture. *Niels .J. Olesen, Helle F. Skall,Britt B. Jensen, Niels H. Henriksen, Stig Mellergård and Henrik Korsholm*
11. Inter-laboratory proficiency tests on detection of notifiable fish diseases. *Niels .J. Olesen, Niccoló Vendramin, Anemone Ojala, Susie S. Mikkelsen*
12. Quality Assurance according to ISO 17025 at Fish Diagnostic Laboratories in Europe: Practice and status. *Olga LM Haenen, Niccoló Vendramin,Anemone Ojala and Niels J. Olesen*

9th International Symposium on Viruses of the Lower Vertebrates:

13. Virus Evolution and Differentiation in Fish Farming, *N.J. Olesen* (keynote)
14. Molecular Tracing of Aquatic Viruses - MOLTRAQ, *S. S. Mikkelsen, H. Schuetze, H. Korsholm, B. B. Jensen, M. S. Bruun, N.J. Olesen.*
15. Inactivation of VHSV by Infiltration and Salt under Experimental

Conditions, *H.F. Skall,C. Jørgensen & N. J. Olesen*

16. Virulence determinant of viral hemorrhagic septicemia virus genotype III isolate for rainbow trout *Oncorhynchus mykiss, T. Ito, J. Kurita, K. Mori and N. J. Olesen*
17. *S.H. Kim, B.J. Thu, H.F. Skall, N. Vendramin and Ø. Evensen*. A single amino-acid located in the CR IV of RNA polymerase of marine viral haemorragic septicaemia (VHSV) determines the in vitro virulence to rainbow trout gill epithelial cells (GECS)

3rd Global Conference of OIE Reference Centres - Incheon (Seoul), Korea, 14–16 October 2014, Aquaculture session

18. Validation on tests, *Niels Jørgen Olesen*

Peer reviewed publications

Peer reviewed publications

1. *Britt Bang Jensen, Annette Kjær Ersbøll, Henrik Korsholm, Helle Frank Skall and Niels Jørgen Olesen* (2014) Spatio-temporal risk factors for Viral Haemorrhagic Septicaemia (VHS) in Danish aquaculture. Diseases of Aquatic Organisms 109: 87–97
2. *Nina Sandlund, Britt Gjerset, Øivind Bergh, Ingebjørg Modahl, Niels Jørgen Olesen, Renate Johansen.* (2014) Screening for Viral Hemorrhagic Septicemia Virus in Marine Fish along the Norwegian Coastal Line. PLoS ONE 9(9): e108529. doi:10.1371/journal.pone.0108529
3. *Laure Bellec, Joelle Cabon, Sven Bergmann, Claire de Boisséson, Marc Engelsma, Olga Haenen, Thierry Morin, Niels Jorgen Olesen, Heike Schuetze, Anna Toffan, Keith Way, and Laurent Bigarré* (2014) Evolutionary dynamics and genetic diversity from three genes of Anguillid Rhabdovirus. Journal of General Virology (2014), 95, 2390–2401
4. *Kim, Sung-Hyun; Thu, Beate J.; Skall, Helle Frank; Vendramin, Niccolò; Evensen, Oystein.* A Single Amino Acid Mutation (I1012F) of the RNA Polymerase of Marine Viral Hemorrhagic Septicemia Virus Changes In Vitro Virulence to Rainbow Trout Gill Epithelial Cells. Journal of Virology, Vol. 88, No. 13, 2014, p. 7189-7198

1.

Participation in international scientific collaborative studies

Participation in international scientific collaborative studies

MOLTRAQ: Molecular tracing of aquatic animal diseases

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

The purpose of the project is 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 will also sequence a large number of isolates ourselves. Data will be used to generate phylogenetic trees and to identify important factors in the evolution and spreading of viruses.

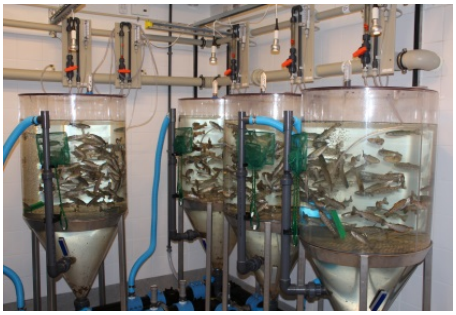
Furthermore, all isolate and genetic information on VHSV and IHNV is uploaded to the EURL database for fish pathogens: www.fishpathogens.eu.

Further information is available at the public project website

www.moltraq.wordpress.com.

VHSV Virulence determinants

A collaborative project to assess putative determinants in VHSV responsible for causing high virulence in fish. Partners: Tamaki Laboratory, Aquatic Animal Health Division, National Research Institute of Aquaculture, Fisheries Research Agency, 224-1 Hiruda, Tamaki, Mie, Japan and Aquatic Animal Health Division, National Research Institute of Aquaculture, Fisheries Research Agency, 422-1 Nakatsuhamaura, Minami-Ise, Mie, Japan



Annex 1 Reagents supplied by the EURL-Fish in 2014

Country	Name	Material	Type	Amount	Date of shipment
Iceland	Sigridur Gudmundsdottir	Mab anti VHSV 1P5B11 anti IPN-Ab – F48 anti IPN-Sp – F51 ISAV Glesvær/2/90 Ampoule VI PT 2013 ISAV FO/01/01/HPR13 Ampoule IX PT 2014	Mab Pab Virus	3 vials, 2 ampoules	18.12.2014
Germany	Omid Taghavian	anti IPN-Ab – F71 anti IPN-Sp – F68	Pab	2 vials	18.12.2014
Greece	Athanasios Prapas	GF cell	Cells	2 x 2 small flasks	05.11.2014
Sweden	Charlotte Axen	Hirame Rhabdovirus: - Polish isolate - Japanese Isolate HRV-8401H	Viruses	2 vials	29.10.2014
Italy	Anna Toffan	Red sea Bream Iridovirus Japanese Isolate from Nakajima	Virus	1 vial	13.10.2014
Sweden	Charlotte Axen	SAV II SDV, SAV-I PDV Hirame Rhabdovirus from Thailand	Viruses	3 x 1,5 ml	13.10.2014
China	Hong Liu	VHSV Ia DK 5151 VHSV ib DK1p8 VHSV III DK 4p101 VHSV IVa US-RBV VHSV IVb US-budd lake	Viruses	5 vials	7.10.2014
South Korea	Hyoung Jun Kim	VHSV Ia DK 5151 VHSV ib DK1p8 VHSV III DK 4p101 VHSV IVa US-RBV VHSV IVb US-budd lake	Viruses	5 vials	7.10.2014
Estonia	Triin Tedersoo	BF-2 and EPC cells	Cells	2 x 2 small flasks	22.9.2014
Ireland	Neil Ruane	ASK1	Cells	2 small flask	4.9.2014
India	J.K. Jena	VHSV Ia DK 5151 IPNV Sp	Viruses	2 vials of each	4.9.2014
Switzerland	Thomas Wahli	Protein A purified anti IPN-Ab F48 + IPN Sp F51	Pab	1 vial	14.8.2014

Country	Name	Material	Type	Amount	Date of shipment
Ireland	Neil Ruane	ASK1	Cells	2 small flask	4.9.2014
USA. Florida	Thomas Waltzek	12 ranaviruses isolates	Viruses	2 ampoules and 10 vials	30.6.2014
France	Michel Bremont	Mab anti IHNV Hyb 136-3	Mab	2 vials (1 concentrated, 1diluted)	30.6.2014
Slovenia	Vlasta Jencic	Mab anti trout IgM 4c10 Pab anti trout IgM k79	Mab and Pab	2 vials	30.6.2014
Iran	Mohades Ghasemi	ASK-1, SSN-1, BF-2 cell lines Pab anti TIV F62 Pab anti SVCV F76 Pab anti SVCV F77	Cell lines and Pab	2 small flasks per cell lines 1 vial per PAb	6.6.2014
Poland	Marek Matras	CCB	Celli lines	1 small flask	4.6.2014
Finland	Riikka Holopainen	KHV TP 30	Virus	3 freeze dried ampoules	30.4.2014
Greece	Athanasios Prapas	BF-2, EPC, CCB	Cell lines	2 small flasks per cell line	30.04.2014
Iceland	Sigridur Gudmundsdottir	SAV Proficiency Test	Viruses	3 vials	3.4.2014
Poland	Marek Matras	EPC, BF-2, RTG2, FHM	Cell lines	2 flasks per cell line	25.3.2014
Norway	Monika Hjortaas	Tissue material from SPF fish	Pathogen free tissue material	10 vials	4.3.2014
Ireland	Neil Ruane	*Aphanomyces invadans* NJM 9701 Ampoule X PT 2013	*A.invadans*	1 freeze dried ampoule	4.3.2014
Faroe Islands	Debes Christiansen	*Aphanomyces invadans* NJM 9701 Ampoule X PT 2013	*A.invadans*	1 freeze dried ampoule	18.2.2014
Serbia	Vladimir Radosavljevic	BF2 and EPC	Cell lines	1 small flask per each cell line	15.1.2014
Sweden	Eva Blomkvist	Pab anti VHSV K930 Pab anti IHNV F63 Pab anti IPNV-Ab F72 Pab anti IPNV-Sp F68 Pab anti SVCV F75	Pab	1 vial per ampoule	7.1.2014

Annex 2 Reagents received in 2014

Country	Name	Institute	Date of receipt	Material	Amount	Protocol no
Slovenia	Vlasta Jencic	National Veterinary Institute	15-01-2014	Rainbow trout, pool of kidney tissue for BKD examination	1	2014-50-2
France	Pierre Boudinot	INRA	08-04-2014	Rainbow trout sera from experimental infection for detection of antibodies against VHSV	88	
Sweden	Eva Blomkvist	National Veterinary Institute	11-04-2014	Salmon, organ material, testing for ISAV and SAV	4	2014-50-40
Norway	Øystein Evensen	Norwegian Life Science University	30-04-2014	Purified RNA from cell culture with IPN, PCR analysis for VHSV, IHNV and SVCV.	2	14-538
Norway	Øystein Evensen	Norwegian Life Science University	9-05-2014	Purified RNA from cell culture with IPN, PCR analysis for VHSV, IHNV and SVCV	4	14-1143
Croatia	Snjezana Zrncic	Veterinary Institute	21-05-2014	Rainbow trout, Supernatant for Virus examination and sequencing	2	14-1731
Croatia	Snjezana Zrncic	Veterinary Institute	21-05-2014	Rainbow trout, Supernatant for Virus examination and sequencing	2	14-1732
Croatia	Snjezana Zrncic	Veterinary Institute	21-05-2014	Rainbow trout, Supernatant for Virus examination and sequencing	2	14-1733
Croatia	Snjezana Zrncic	Veterinary Institute	21-05-2014	Sea bass, brain tissue for Virus examination/noda virus suspicion	2	14-1734
Croatia	Snjezana Zrncic	Veterinary Institute	03-06-2014	Sea bass, tissue fixed and embedded in paraffin for histopathology, Rickettsia suspicion	3	14-2578
Sweden	Charlotte Axen	National Veterinary Institute	10-06-2014	Rainbow trout, Supernatant and organs for SAV PCR	7	14-2783

Country	Name	Institute	Date of receipt	Material	Amount	Protocol no
Iran	Mohades Ghasemi	Inland Water Aquaculture Institute	02-06-2014	Rainbow trout, Grey Mullet, Carp, Angel fish, Sturgeon, organs, supernatant etc. for virological examination, PCR etc.	18	14-2873
Serbia	Vladimir Radosavljevic	Institute of Veterinary Medicine of Serbia	03-06-2014	Rainbow trout, spleen, heart, kidney for SAV detection and sequencing; Ameiurus (Ictalurus) nebulosus, supernatant for Rana virus detection and sequencing.	5	14-2965
Sweden	Charlotte Axen	National Veterinary Institute	03-06-2014	Crucian carp, Rainbow trout	6	14-3006
Serbia	Vladimir Radosavljevic	Institute of Veterinary Medicine of Serbia	03-06-2014	Carp, gill and kidney tissue for KHV confirmation and sequencing	5	14-9691
Iran	Mehdi Khalaj	Islamic Republic of Iran Veterinary Organization	08-09-2014	Rainbow trout, 5 x supernatant, 7 x organ material for virological examination and PCR	12	14-9060
Croatia	Snjezana Zrncic	Veterinary Institute	03-06-2014	Sea bass, brain tissue for bacteriological and virological examination and brain tissue fixed in formaldehyde for histopathology, Rickettsia/noda virus suspicion	14	14-15924
Norway	Monika Hjortaas	Norwegian Veterinary Institute	12-12-2014	Rainbow trout, Blood pellet, Virus Y, positiv control material for PCR	2	14-16130