

# **Report of the**

# 26<sup>th</sup> Annual Workshop of the National Reference Laboratories for Fish Diseases

Kgs. Lyngby, Denmark 30<sup>th</sup> and 31<sup>st</sup> of May 2022



Organized by the European Union Reference Laboratory for Fish and Crustacean Diseases, National Institute of Aquatic Resources, Technical University of Denmark, Kgs. Lyngby

# Contents

Introduction and short summary	3
Programme	5
SESSION I: Update on important fish diseases and their control	7
Overview of the fish diseases situation and surveillance in Europe in 2021	8
Overview of the disease situation in Norway in 2021	
Epidemiological investigation of the Infection Haemopoetic Necrosis (IHN) outbreak occurred in Denmark in 2021	11
First outbreak of IHN in Denmark	
IHN in Denmark and the way forward	
IHNV outbreak in Finland in 2021	14
Infectious salmon anemia outbreak in farmed Atlantic salmon ( <i>Salmo salar</i> L.) in Iceland, first detection of an ISAV HPRdel variant	15
SESSION II: Control and Surveillance of fish diseases in EU	16
Sanitary situation in France: From IHN and VHS eradication to the characterization of unknown and emergent viruses	17
Viral Hemorhagic Septicaemia outbreaks in Rainbow Trout (Oncorhynchus mykiss) in Romania	
Characterization of a novel infectious pancreatic necrosis virus (IPNV) genogroup 6 identified in lake Vänern, Sweden	19
Health status of farmed cyprinids in the Czech Republic	
SESSION III Results from ongoing research on listed and emerging fish diseases	21
Prevention of PD in Atlantic salmon by vaccination – experience from field	
Effect of Water Temperature on the Infection Kinetics of <i>Piscine orthoreovirus</i> Genotype 3 in Rainbow Trout	23
First detection of Cyclopterus lumpus virus (CLuV) in England, following a mortality event	
Overview of the OIE Collaborating Centre for Emerging Aquatic Animal Diseases	
SESSION IV:Update from the EURL	26
EURL Training Courses for 2022	
2022 Inter-laboratory proficiency test for fish diseases	
EURL for Fish Diseases, work done in 2021	
Workshop evaluation	30
Greetings and conclusions of the meeting	38

# Introduction and short summary

The 26<sup>th</sup> Annual Workshop of the National Reference Laboratories for Fish Diseases was held on May 30<sup>th</sup> and 31<sup>st</sup> 2022.

Because of the Covid-19 pandemics and the limitation to travel to and from Denmark the workshop was held in hybrid form; there were 35 participants who attended in person the meeting (21 from out of Denmark) and more than 60 registered to attend online.

The virtual organization of the meeting has allowed a significant expansion of the number of participants attending the workshop as well as the number of oversea countries participating.

The number of participants has reached 95 participants from 38 countries attending over the two days period. There were four sessions with a total of 18 presentations.

The workshop was held back to back with the 13<sup>th</sup> Annual Workshop for National Reference laboratories for crustacean diseases and a special session for NRL in EU and EEA on the implementation of the Animal Health Law.

The scientific programme of the Annual Workshop was again this year wide and covered many interesting topics.

The workshop was opened with "Welcome and announcements" by Head of the EURL for fish diseases, Niels Jørgen Olesen. The scientific part was opened with the traditional Session 1 "Update on important fish diseases and their control", in which participants had the opportunity to present new findings from their respective countries.

Initially, an overview of the disease situation and surveillance in Europe 2021 was provided on the basis of the results obtained from the Survey & Diagnosis questionnaire. A report compiling all information is available at the EURL website <u>https://www.eurl-fish-crustacean.eu/</u>.

Secondly, the fish disease situation in Norway was presented; a detailed report (currently in Norwegian, soon in English) is available at <u>https://www.vetinst.no/rapporter-og-publikasjoner/rapporter/2022/fiskehelserapporten-2021</u>

Due to the occurrence of the first outbreak of Infectious Haematopoietic Necrosis (IHN) in Denmark in 2021, four presentations on this topic followed the overview of the fish health report in Norway. The first presentation was given by Johanne Joey Ellis-Iversen and Argelia Cuenca who described the epidemiological investigation and the phylogenetic analysis to trace the infection. Afterwards Niccoló Vendramin provided insights on the laboratory findings and investigation conducted by the Danish NRL and EURL for fish disease on the IHN isolate. Prof. Niels Jørgen Olesen provided an overview of the plan to control the disease in Denmark. The representative of the Finnish NRL Tuija Kantala provided an overview of the IHN outbreak occurred in Finland, after introduction of infected fish from Denmark. The session finished with a presentation on ISA outbreak occurred in Iceland by Heiða Sigurðardóttir.

After a short break, session II Control and Surveillance of fish diseases in Europe.

This session consisted of four talks. The first presentation given by the representative of the French NRL Lenaig Loubotin, describing the sanitary situation in France, encompassing the eradication plan for VHS and IHN, as well as characterization of novel pathogen in Gilthead sea bream. Afterwards, the representative of the Romanian NRL, Mihaela Costea, gave online a presentation on VHS outbreak in Romania. The investigation on pathogenicity to rainbow trout, salmon and brown trout of IPNV genogroup 6 was given by representative of Swedish NRL, Charlotte Axen. Finally,

researcher Dr. Lubomir Pojezdal gave an update on infectious disease affecting cyprinids farming in Czech republich. The first day of the meeting was then finalized, participants attending the meeting in Denmark were transferred then to Tivoli for social dinner.

The second day Session III took place with presenting results from ongoing research on listed and emerging fish diseases.

The session started with an presentation from Dr. Britt Bang Jensen in experiences gathered from field testing of DNA vaccine for protecting Atlantic salmon from PD in Norway.

Then the PhD student at DTU AQUA Juliane Sørensen, presented the results of the outcome of PRV-3 infection in rainbow trout at three different temperatures. The session was concluded by Richard Paley from the OIE collaborating center for emerging aquatic animal diseases, presenting the activity of the center as well as the description of a recent outbreak of Flavivirus in lumpfish.

After a short coffee break, Session IV Update from the EURL for fish diseases took place.

In this session the EURL training courses scheduled for October 2022 were advertised. A resume of the InterLaboratory Proficiency test 2021 was presented by Teena Vendel Klinge, summing up the results of the online workshop where results of the ILPT were presented and discussed by all participants, as well as the feedback on ILPT 2021 were presented. Furthermore the EURL activities in year 2021 were presented and proposals for the EURL work plan for 2021and 2022 were discussed. It was informed that the work plan will include tasks for both fish and crustacean diseases.

Employees from DTU Aqua took minutes from the meeting: Argelia Cuenca, Therese Birgitte Christoffersen, Niccolò Vendramin and Morten Schiøtt. Niccolò Vendramin assembled the report. Albeit there were some technical issues that challenged the normal flow of the workshop, we regard this activity as a success and a great venue for knowledge sharing.

We would once again like to thank all the presenters for their great contribution, without them the meeting would not have been a success. The workshop and meeting was organized by a team consisting of Morten Schiøtt, Niccoló Vendramin and Niels Jørgen Olesen, with the help from the rest of the fish disease section at the National Institute of Aquatic Resources, DTU AQUA. The meeting next year is tentatively planned to be held at end of May 2023, in a face to face meeting at DTU Aqua. More details will follow.

We wish to thank all of you for participating and we are looking forward to seeing you next year.

Niels Jørgen Olesen and Niccolò Vendramin

# Programme

# Monday May 30<sup>th</sup> Annual Workshop of the National Reference Laboratories for Fish Diseases

13:00 - 13:10	Welcome and announcements Niccoló Vendramin and Niels Jørgen Olesen
SESSION I:	<b>Update on important fish diseases and their control</b> <i>Chair: Niels Jørgen Olesen and minutes: Morten Schiøtt</i>
13:10 - 13:30	Overview of the disease situation in Europe Niccoló Vendramin
13:30 - 13:50	Overview of the disease situation in Norway Torfinn Moldal
13:50 - 14:20	Epidemiological investigation of IHN outbreak in DK Johanne Joey Ellis-Iversen, Argelia Cuenca
14:20 - 14:40	IHN outbreak in DK – laboratory Tine Moesgaard Iburg; Niccoló Vendramin
14:40 - 14:50	– and the way forward Niels Jørgen Olesen
14:50- 15:05	IHN outbreak in Finland? <i>Tuija Kantala</i>
15:05 - 15:25	ISA outbreak in Iceland – <i>Heiða Sigurðardóttir</i>
15:25 - 15:50	Coffee break
SESSION II:	<b>Control and Surveillance of fish diseases in Europe</b> <i>Chair: Niccoló Vendramin and minutes: Argelia Cuenca</i>
15:50 - 16:10	Sanitary situation in France: from IHN and VHS eradication plan to the characterization of unknown and emergent viruses <i>Lenaig loubotin</i>
16:10 - 16:25	VHS in Romania Mihaela Costea
16:25-16:40	IPNV genogroup 6 pathogenicity to rainbow trout, salmon and brown trout <i>Charlotte Axen</i>
16:40 - 16:55	Health status of farmed cyprinids in Czech republic Lubomír Pojezdal

16:55-17:10 End of the day – summing up and announcements

Bus transfer to town

# Tuesday May 31rd 26<sup>th</sup> Annual Workshop of the National Reference Laboratories for Fish Diseases

SESSION III:	<b>Results from ongoing research on listed and emerging</b> <b>fish diseases</b> <i>Chair: Morten Schiøtt and minutes: Niccoló Vendramin</i>
10:00 - 10:10	Welcome and announcements
10:10 - 10:30	Prevention of PD in Atlantic salmon by vaccination – experience from field - Britt Bang Jensen
10:30 - 10:50	Effect of temperature on PRV-3 infection in Rainbow trout Juliane Sørensen
10:50 - 11:05	An outbreak of Lumpfish Flavivirus, diagnostic and control <i>Richard Paley</i>
11:05-11:20	OIE collaborating centre for emerging aquatic animal disease <i>Richard Paley</i>
11:20- 11:40	Coffee break
SESSION IV:	Update from the EURL for fish diseases
11:40 - 11:55	EURL Training Courses. Topics and organization of courses 2022 Niccoló Vendramin and Tine Moesgaard Iburg
11:55 – 12: 10	Interlaboratory Proficiency test for fish diseases 2021 Teena Vendel Klinge and Niccoló Vendramin
12:10 - 12:30	EURL Work done in 2021, plan for 2022 and ideas and plans for 2023 Niels Jørgen Olesen Next meeting and end of 26 <sup>th</sup> Annual Workshop Niels Jørgen Olesen

## END OF FISH WORKSHOP

SESSION I: Update on important fish diseases and their control Chair: Niels Jørgen Olesen

# Overview of the fish diseases situation and surveillance in Europe in 2021 Niccolò Vendramin, Teena Vendel Klinge and Niels Jørgen Olesen

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#### Abstract

This report is based on the data from the questionnaire on Survey and Diagnosis of the listed fish diseases in This report is based on the data from the questionnaire on Survey and Diagnosis of the listed fish diseases in Europe (S&D) for 2021. The Questionnaire is provided by the EU Reference Laboratory for Fish and Crustacean Diseases, it is collated annually and is the only comprehensive overview of the disease situation in fish farming in Europe. The information has been made available on the EURL web site (<u>www.eurl-fish-crustacean.eu</u>), where all raw data can be obtained. The questionnaire comprises 4 parts:

- 1. General data on aquaculture fish production: Number of fish farms, and their health status according to AHL 2016/429, and information on national surveillance programmes.
- Epidemiological data on the disease situation in each Member State with focus on the listed diseases (information on number of out breaks and increase or decrease in number of infected farms and severity of outbreaks) but also including other diseases of interest.
  Laboratory data from the NRLs and other laboratories, including the numbers of samples examined, and diagnoses of fish diseases
- Laboratory data from the NKLs and other faboratories, including the numbers of samples examined, and diagnoses of fish diseases made.
  A Nuclear the state of the laboratories in the state of the state
- 4. A National report describing health and surveillance situation in general. These reports are compiled into one and can be found on <a href="https://www.eurl-fish-crustacean.eu/">https://www.eurl-fish-crustacean.eu/</a>.

#### **Production data for 2020**

The most updated data on aquaculture production in Europe refer to 2020 on the website of Federation of European Aquaculture producers. We decided to refer to the dataset provided by <u>FEAP</u>. At global level, the pandemic is considered to affect significantly aquaculture production. According to FAO assessment, the Covid-19 pandemics caused an estimated drop in overall output of 1.3 percent in 2020. According to data provided by FEAP, there has been a reduction in the growth of the Aquaculture sector in Europe. The total fish production in aquaculture in Europe, including Turkey and Norway, increased slightly from 2019 and is now at 2,570,650 t. The total production of EU countries has reduced in 2020 from 515,946 tonns to 505,734 tonns.

#### Number of fish farms in Europe

The total number of authorised/licensed fish farms in Europe is estimated in about **36239** farms. This number is a rough estimate as three participants did not provide updated numbers,

Germany provides the largest contribution when it comes to fish farm with 13231 farms, while the second contributor is Poland with 5191. In both cases, this high nubmer of farms reflect a large numbr of small size farms.

When it comes to production, Norway has by far the largest production in Europe and has licensed 1258 farms/sites. An overview of the number in each country can be found in Annex 1. It has to be ackowledged that it was not possible to retrieve the total number of active farms for all participants in the survey (3 missing) and therefore nubmer of active farms in 2019 have been used to fill these gaps instaed.

**Health Status of fish Farms**: According to AHL the terminology of health category describe in 2006/88 has been changed to health status. There are currently four possible health statuses:

- 2) Under eradication/control program
- 3) In voluntary surveillance program
- 4) Non approved disease free and not under eradication/control program.

<sup>1)</sup> Approved disease free

In 2021, a health status was assigned to 13787 farms with susceptible species to VHS, to 13710 farms with susceptible species to IHN, to 5679 farms with susceptible species to ISA.

**Health status for VHS**, 11% of fish farms are approved disease free, 3% is under eradication/control program, 11% under voluntary program, 75% is not approved disease free and not under eradication/control program

**Health status for IHN,** 20% of fish farms are approved disease free, 3% is under eradication/control program, 4% under voluntary program, 73% is not approved disease free and not under eradication/control program

Health status for ISA (Infection with HPR $\Delta$  ISAV), 66% of fish farms are approved disease free, 0% is under eradication/control program, 0% under voluntary program, 34% is not approved disease free and not under eradication/control program

## Outbreaks of listed diseases in Europe

Out of 33 participating laboratories, 7 reported an increase in the number of fish farm infected with notifiable diseases.

For VHS, 24 new outbreaks were reported in Europe in 2021 (11 in 2020), 15 of these were in Germany. The remaining outbreaks were observed in Italy (3), Czech republic (2), Austria, Belgium, France, Romania, (1).

For **IHN**, 104 new outbreaks were reported. The majority was in Germany (82). Denmark experienced the first outbreak of IHN, and recorded in 2021 8 infected farms and 3 put and take lakes (11). The remaining outbreaks were in Finland (5), Austria (4), Italy (1). A series of 4 presentation will focus on IHN situation during 26<sup>th</sup> Annual workshop for National Reference Laboratories for Fish Diseases.

For ISA (Infection HPR $\Delta$  ISAV) Iceland reported the first outbreak and Norway reported an increased number of outbreaks compared to 2020 (29). Due to the increase in number of outbreaks 2 specific talks on the topic will be provided at the 26<sup>th</sup> Annual Workshop for National Reference Laboratories for Fish Diseases.

For KHVD, 65 outbreaks were reported in 2021.

#### Other fish diseases problems in Europe

A whole range of other disease problems in 2021 were reported:

- In **rainbow trout** the major concerns remain flavobacteriosis/RTFS (*F.psychrophilum*), Enteric Redmouth Disease (*Y. ruckeri*), Furunculosis (*A. salmonicida*), Bacterial kidney disease (*R.salmoninarum*), and Infectious Pancreatic Necrosis, with an increase of clinical outbreaks.
- In **salmon** farming the major concern is sea lice; after the ectoparasite a number of disease problems cause concerns and includes pancreas disease, heart and skeletal muscle inflammation, cardiomyopathy syndrome, amoebic gill disease and complex gill disease CGD (amoebic gill disease, salmon gill poxvirus, *Paranucleospora theridion* etc.), infection with *Piscirickettsia salmonis*.
- In **Cyprinid** it is primarily CEV.
- In European seabass and Gilthead seabream it is primarily VNN/VER, *Photobacterium damselae* subsp. *Piscicida*, tenacibaculosis, *Vibrio harvey*, *Sparicotyle chrysophrii* infection.

Q: Has there been an increase in severity of outbreaks of CEV?

A: No.

## **Overview of the disease situation in Norway in 2021**

Torfinn Moldal, Ingunn Sommerset, Cecilie S. Walde, Britt Bang Jensen, Jannicke Wiik-Nielsen,

Geir Bornø, Victor Henrique Silva de Oliveira, Asle Haukaas and Edgar Brun

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#### Abstract

The 'Norwegian Fish Health Report' has been published by the Norwegian Veterinary Institute annually since 2003. The report focuses on health and welfare of farmed fish, but wild fish are also included. Since 2020, the Norwegian Veterinary Institute has gained access to data at site level for several non-notifiable diseases from private diagnostic laboratories. The agreements cover the majority of active sites. With these data, the prevalence and geographical distribution of important non-notifiable diseases, such as heart and skeletal muscle inflammation (HSMI) and cardiomyopathy syndrome (CMS), are reported in a more representative way. The report for 2021 in Norwegian was published in March, and the English version is expected to be published in June on <a href="https://www.vetinst.no/rapporter-og-publikasjoner/rapporter">https://www.vetinst.no/rapporter-og-publikasjoner/rapporter</a>.

The main species in Norwegian aquaculture is Atlantic salmon. In 2021, more than 54 million farmed salmon died during the seawater phase of the production. The probability of a fish dying during the year was 15.5%, and has varied from 14.7 to 16.1% in the last five years. As previous years, the high mortality in the seawater phase have multiple and complex causes. The main reasons for mortality are reported by fish-health personnel to be mechanical injuries after delousing operations, cardiomyopathy syndrome (CMS) and infections caused by *Moritella viscosa* and *Tenacibaculum* spp. There are still geographical differences with mortality over 20% in the western part of Norway and less than 14% in the very south and from mid-Norway and northwards. Mortality data for cleaner fish used for biological delousing are incomplete, but previous reports have indicated a near total mortality of cleaner fish throughout the production cycle.

Among the notifiable viral diseases, the infectious salmon anemia (ISA) is still a concern with 25 confirmed outbreaks and detection of virulent ISA-virus at additionally four sites in 2021. One outbreak was at a broodstock site, one outbreak was in a hatchery, and the remaining 23 outbreaks were at sea sites with grow-out fish. Fourteen of the sites were covered by existing restriction zones for ISA at the time of ISA suspicion, with four located in a protection zone and ten located in a surveillance zone. The fish were reported to have been vaccinated against ISA at two of the sites. Since 2019, a surveillance programme for ISAV HPR0 has been underway in hatcheries. Around half of the Norwegian hatcheries are tested for ISAV HPR0 at one sampling every second year. In 2021, eight out of 78 hatcheries in the surveillance programme tested positive for ISAV HPR0.

Regarding pancreas disease (PD), 100 outbreaks were registered in 2021, compared with 158 the year before. The reduction could be related to a decrease in SAV3 cases in production area 5 and SAV2 cases in production area 6. As in 2020, no outbreaks were reported in the surveillance zone outside the endemic area.

A risk-based surveillance programme for IHNV and VHSV based on examination of samples submitted for routine diagnostic investigation is in place. In 2021, wild pink salmon, brown trout in cultivation and grow-out sites as well as rainbow trout in inland sites were also included in the surveillance programme. Neither IHNV nor VHSV was detected in Norway in 2021.

Q: How do you see the ISA control program in the future (more vaccination?)

A: Aim is less than 10 outbreaks per year and a three fold increase in vaccine doses

# Epidemiological investigation of the Infection Haemopoetic Necrosis (IHN) outbreak occurred in Denmark in 2021

#### Johanne Ellis-Iversen<sup>1</sup> and Argelia Cuenca<sup>2</sup>

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#### Abstract

On the 18/5/21 Denmark declared its first outbreak of IHN ever. Denmark was previously declared free from IHNV and had surveillance in place according to EU Regulation 2021/620. By end of September 11 cases of IHNV were declared – eight in fish farms and three in fishing lakes. The EURL at DTU AQUA investigated the molecular epidemiological links between the Danish IHNV isolates and other isolates which sequence are either available in public databases or at the EURL sequence repository. DTU Aqua carried out a detailed molecular investigation into the connection and genetic evolution of the Danish strains during the outbreak including comparison of whole genome sequences between the farms and over time. At the same time the Danish Veterinary and Food administration carried out field epidemiology investigations to identifying likely transmission routes between outbreaks with the aim to reduce spread of the disease. All investigations are still ongoing and we will present the latest updates and combined conclusions at the workshop.

*Q*: Have you had the opportunity to compare the sequence of the IHNV isolated in Denmark with sequences from IHNV strains detected in Germany?

A: The sequences from 2021 has been received and analysed, the ones from 2015 - 2021 are in process of being received.

# First outbreak of IHN in Denmark

### Niccoló Vendramin, Argelia Cuenca, Tine Iburg Niels Jørgen Olesen

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Infectious hematopoietic necrosis virus was detected for the first time in Denmark, 17<sup>th</sup> of May 2021. Along with the epidemiological surveillance for monitoring and controlling the spread of the disease in the country a series of technical and scientific studies have been carried out by the team of Unit for fish and shellfish diseases in order to characterize the new virus isolate and assess and improve diagnostic procedures and detection methods.

This presentation will compile four main topics:

- 1) The modification of the RT-qPCR assay based on a new probe designed by Hoferer et al.,2019 made available from the NRL for fish diseases in Germany for detection of different variants of IHNV circulating in Europe
- 2) The investigation of what organs are most suitable for increasing the capacity of detecting IHNV during different phases of infection in fish tissue
- 3) A virulence trial assessing the capability of infecting and reducing survival of two main salmonids species (Rainbow trout and Atlantic salmon)
- 4) The persistence of IHNV in infected population under field condition in a farm placed in restriction zone with changing water temperature

The presentation will compile methodology and results.

Hereunder the main conclusions:

- The new probe proposed by Hoferer et al.,2019 has replaced the previously recommended one. The new method has been included in the diagnostic manual and information has been circulated to the NRL network both via e-mail and the EURL website <u>https://www.eurl-fishcrustacean.eu/news/nyhed?id=3ad8a31a-1412-45a7-b96b-53a930f99bcc</u>
- 2) Testing separately pool of organs (spleen, heart and kidney) and brain has shown that testing brain does not significantly increase the capability of detecting IHNV in the epidemiological surveillance conducted in connection with the IHN outbreak
- 3) The new isolate appears to infect more efficiently Rainbow trout than Atlantic salmon and induce significant reduction of survival compared to a reference IHN isolate
- 4) No apparent reduction in the capability of detecting the virus was observed at temperatures above 14°C and the observed reduction in relative number of positive samples was attributed to infection kinetics at the farms and not the temperature

Hoferer, M.; Akimkin, V.; Skrypski, J.; Schütze, H.; Sting, R. Improvement of a diagnostic procedure in surveillance of the listed fish diseases IHN and VHS. J. Fish Dis. 2019, 42, 559–572.

## IHN in Denmark and the way forward

#### Niels Jørgen Olesen, Niccolò Vendramin, Argelia Cuenca, Tine M. Iburg

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Denmark lost its status as IHN free Member State December 10<sup>th</sup> 2021. By then 28 of 163 farms with susceptible species obtained approval as IHN free compartments.

By March 2022 all except three IHN infected farms have emptied, disinfected and fallowed the farms before restocking.

IHN detected again April 20<sup>th</sup> at Simested Å on not previously infected farm. Epidemiological investigations was initiated, and contact farms were identified and sampled for laboratory examination.

Eight farms were following found positive (by May 24<sup>th</sup>) all either infected by trade or waterborne infection. One farm infected in 2021 got reinfected by trade in 2022. Further epidemiological examination and molecular tracing is awaiting.

A survey to assess the de-facto spreading of IHN in Denmark was initiated in April/May 2022 comprising 40 high risk farms. These farms are inspected and sampled by designated vets.

In case of clinical signs the CA are notified for official sampling.

Based on the survey an eradication program according to (EU) 2020/689 will hopefully start end of 2022. All IHNV infected farms and farms with inlet water having passed infected farm shall drain, disinfect and fallow for 6 weeks (synchronized 3 weeks) and restocked with certified IHNV free fish. If possible combined with electrofishing removing all rainbow trout.

The control program will comprise all Danish fish farms except the 28 IHNV free compartments. Denmark will possibly be divided in approx. 20 zones and a 4-year program initiated in each zone. The surveillance will be based on cell culture and with rapid RT-qPCR in suspect cases. New outbreaks should be followed by immediate eradication.

Denmark will putative regain IHN free status in 2027.

No vaccines are on the market yet and when it comes, it will be expensive. No compensation from the government. No insurance scheme and no "Trout Fund" to compensate infected fish farms

It is doubtful whether fish farming can live with IHN in Denmark and at the same time be profitable. The industry and DK have previously shown that it is possible to eradicate VHS, it must be possible to do it again.

*Q*: 20 eradication zones – how is trade between these zones, are these zones able to trade without testing?

*A*: yes it will be possible – in case of outbreak the zones can be closed down.

*Q*: how was virus introduced into DK? From import of fish or from wild fish?

A: probably through trade with Germany – after export to Germany, trucks may have been infected and brought back virus if disinfection of truck was not efficiently performed. Marine farm bought fish from infected farm.

Q: Are wild fish being monitored?

*A:* Only for restocking program on Atlantic salmon. In connection to recent outbreak, electrofishing around infected farms showed no Rainbow trout and no infected wild fish.

# IHNV outbreak in Finland in 2021 Tuija Kantala and Tuija Gadd

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#### Abstract

In 2021, IHNV was detected in five fish farms in Åland Islands. The infection had spread from Denmark via fish movements during the spring. Danish animal health authorities notified Finnish colleagues about an epidemic in Denmark and fish movements from the infected sites to two sea-cage fish farms in Åland Islands. The first farm was then inspected and sampled on May 24<sup>th</sup>. No abnormal mortality or symptoms had been reported. During the inspection, however, some abnormally swimming fish were detected. In autopsy, the fish showed findings related to IHNV, including hemorrhages in skin and muscle. The organ samples arrived in the virology laboratory on May 25<sup>th</sup>, and IHNV was detected in pooled organ suspensions by real time RT-PCR on May 26<sup>th</sup>. On May 27<sup>th</sup>, sequencing further confirmed that the virus was IHNV. In addition, cytopathic effect typical for IHNV was detected on both EPC and BF cell lines, and IHNV was confirmed by ELISA. The second farm was inspected and sampled on May 26<sup>th</sup>, and the presence of IHNV was confirmed on June 1<sup>st</sup>.

Epidemiological investigation was carried out to find out where the infection had possibly been spread in Finland. A third sea-cage farm, located approximately 2 km from the first two infected farms, was found positive on June 4<sup>th</sup>. The infection had probably spread there via water from the first two farms. After the summer, IHNV was further detected in two more farms: in the fourth farm on October 5<sup>th</sup>, and in the fifth farm, on October 22<sup>nd</sup>. These two farms located further (approximately 6-20 km) from the first three positive farms. It is not clear how the virus spread to these farms, but transmission especially via boat traffic has been suspected. A restricted zone was immediately established around the first infected farms and later extended to cover each of the IHNV positive farms. All the IHN sensitive fish from the infected farms were euthanized or slaughtered. Sea-cages and other equipment from the farms were washed, disinfected, and after disinfection, a fallowing period of at least six weeks was carried out. In practice, last rainbow trouts were slaughtered just before Christmas. A total of approximately 500 000 kg of rainbow trout were euthanized, and 900 000 kg were slaughtered. Eradication was completed in March 2022. The first new rainbow trouts were moved to infected sites in early April. On April 27th, 2022, surveillance program of two years started in the surveillance zone which corresponds to the original restricted zone. Sequencing of partial G (glycoprotein) gene of IHNV genome indicated that the virus belonged to IHNV genotype E. In addition, the Finnish isolates were identical with the Danish isolates. The previous IHNV outbreak in Finland in 2017–2018 was caused by IHNV that was closest to genotype U. Currently, Finland is officially declared free of IHN, with exception of the coastal compartment corresponding the surveillance zone in Åland Islands. After the 2017–2018 IHN outbreak, the surveillance program in those zones was completed and the areas were declared free of IHN on September 15<sup>th</sup>, 2021.

Q: When eradication program started, did the Finnish government give compensation to farmers?

A: Some compensation was provided

# Infectious salmon anemia outbreak in farmed Atlantic salmon (*Salmo salar* L.) in Iceland, first detection of an ISAV HPRdel variant

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### Abstract

Infectious salmon anemia (ISA) is a serious viral disease of Atlantic salmon (*Salmo salar* L.) caused by the ISA virus (ISAV) and is notifiable to the World Organization for Animal Health (OIE). Virulent strains ISAV-HPRdel have deletions in a highly polymorphic region (HPR) of the hemagglutinin-esterase (HE) gene on segment 6, whereas avirulent strains ISAV-HPR0 have none.

Routine targeted samplings for Real-time RT-PCR analyses have been performed since 2009. Icelandic Atlantic salmon broodfish farms are formally declared free of ISA by the fish health authority of the European Union. (https://www.mast.is/static/files/aaetlanir/aquaculture-surveillance-programme-fish-diseases-2020.pdf)

ISAV screening with RT-qPCR started at Keldur in 2011. The few samples that have been ISAV positive from broodfish are HPR0 genotype. A research project carried out at Keldur from 2015-2018 screened both wild and cultured salmon juveniles, cultured salmon in sea pens and wild salmon from rivers (a total of about 800 fish) for ISAV, which was not detected.

In November 2021, an increased mortality was experienced in farmed Atlantic salmon in sea pens in Reyðarfjörður, East Iceland. The fish showed macroscopic clinical signs suggestive of ISAV. Tissue samples and organs were sent to Keldur for diagnosis, using ISAV RT-PCR and histopathological analysis.

The histopathology of the diseased fish was consistent with previous descriptions of ISAV-del infections in Atlantic salmon, i.e., characterized by extensive hemorrhage and congestion in most organs, associated with varying degree, often significant, pathological changes. Erythrophagocytosis was commonly observed, due to extensive immune reaction.

RT-qPCR results for ISAV were positive with Ct. values ranging from 14-27. The samples were also run in a ISAVseg6 RT-PCR and further analysed by capillary electrophoresis. Sequencing of the ISAVseg6 PCR amplicons showed that it was an ISAV HPRdel variant. This is the first time that an HPRdel variant of ISAV has been detected in Iceland. The complete HE-gene was sequenced and when aligned with published sequences it showed greatest similarity to HPR0 and HPRdel sequences from northern Norway and HPR0 sequences from The Faroe Islands.

- Q: What control measures were taken?
- A: Slaughter of fish
- Q: Did the ISA originate from a new HPR deletion?
- A: Maybe, but currently not known.
- Q: Are the fish vaccinated against ISA?
- A: No, not at the moment but maybe they will be

SESSION II: Control and Surveillance of fish diseases in EU Chair: Niccoló Vendramin

# Sanitary situation in France: From IHN and VHS eradication to the characterization of unknown and emergent viruses

### L. Louboutin<sup>1</sup>, M. Baud<sup>1</sup>, L. Bigarre<sup>1</sup>, J.C abon<sup>1</sup>, L. Pallandre<sup>1</sup>, L. Cloastre<sup>2</sup>, G. Le Febvre<sup>3</sup>, I. Guerry<sup>3</sup>,

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#### Abstract

Although our territory contains many zones and compartments free of IHNV and VHSV, these listed Rhabdoviruses have been occasionally detected in France since their first description in 1971 and 1989 respectively. In order to obtain a free status for those diseases, a National Eradication and Surveillance Plan, supported by European Union, was started in France in 2017. The amount of laboratory analysis has been consequently increased these last 5 years, reaching around 700 analysis in 2021. Almost all the metropolitan territory is involved in this approach and more and more sites of production are committing to this program. Moreover, an important effort was done to integrate the characteristics of the extensive aquaculture, mainly represented by carp and pike production in France. Vector species for VHSV and IHNV, pike are mostly imported from the East of Europe, without any certificate to ensure their sanitary status. In order to secure fish trade, French professionals and administration discussed with their counterparts in Czech in last October, in order to identify constructive solutions.

In parallel of this plan, some new viruses are regularly identified and described, mainly thanks to the cell culture-based tools and new generation sequencing. For example, we recently described a new picornavirus isolated from seabream larvae showing an abnormal increase of mortality in early stages of development. The genome of this new virus, proposed to be called *potamipivirus daurada*, was annotated and compared to other Picornaviridae family members and a specific RT-qPCR was developed to screen fish farms.

**Keywords**: Sanitary situation, France, VHS, IHN, National plan for eradication and surveillance, Emerging diseases, Cell culture tools and NGS

Q: Do you screen for pike fry rhabdovirus?

A: the screening on cell culture will detect it.

# Viral Hemorhagic Septicaemia outbreaks in Rainbow Trout (*Oncorhynchus mykiss*) in Romania

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#### Abstract

Romanian trout farms are situated, in generally, in zones appropriate for salmonids rearing, at altitudes of 340-1450 meters. The water source is ensured by river water, natural spring water or artificial lakes. Intensive reared salmonids species, beside rainbow trout (*Oncorhynchus mykiss*), are: brown trout (*Salmo trutta fario*), brook trout (*Salvelinus fontinalis*), grayling (*Thymalus thymalus*) and huck (*Hucho hucho*).

Viral hemorrhagic septicemia virus (VHSV) was diagnosed in Romania for the first time in 2007, followed by another case in 2016.

In November 2021, a mortality started in one rainbow trout farm situated in the Buzau County. At the moment of outbreak, the water temperature was 10 °C. According the weight, in the farm were four categories of fish: trout of 200 g/fish, trout of 50-60 g/fish, fingerlings (10-15 g/fish) and fry.

The disease has started in one basin of farm and rapidly has spread in all populated basins, including the incubation station. Mortality increased in the first thirteen days when registered the pick corresponding at a 0.74% mortality percent.

At the end of January 2022, a mortality started in two close trout farms situated in the Sibiu County, same owner. At the moment of outbreak, the temperature of water was 8-10 °C °C in farm 1 and 6-8 °C in farm 2. Both farms had trout in several age groups. Sturgeons were also raised on the farm 2. The daily mortality in both farms varied from 0.1% to 0.7%.

Specimens with apathy and/or abnormal swimming, skin darkening, exophthalmia and gill palness were taken for laboratory investigations, from all farms. Anatomopathological exam in farm from Buzau County showed bleeding around the eyes and on the pectoral fin bases, paleness or white-grey gill with rare hemorrhages, liquid in the abdominal cavity, discolored liver, hemorrhagic spots on surface of the fatty tissue and on swim bladder, low firmness kidney, hemorrhagic spots in the muscles. Anatomopathological exam in farms from Sibiu County showed excess mucus on the skin and gill, reddenind of mouth corner, palate and on the internal face of operculum, enlarged spleen, discolored liver, hemorrhagic spots on the liver and swim bladder, hemorrhagic spots in muscles.

In all cases, for the virological examination, tissue pools were prepared, triturated and centrifuged according with the working instructions. An aliquot from tissue homogenate were been diluted 1:10 and inoculated on EPC and BF2 cell lines. After the cytopathic effect occurred, ELISA and IFAT tests were performed for VHSV and IHN virus identification. The virological examination showed positive results for VHSV and negative results for IHNV. Supernatant were tested in Romanian NRL by Real Time PCR and sequencing. Viral hemorrhagic septicemia virus was confirmed in both outbreaks.

At the time of infection confirmation with VHS virus, disease control measures were imposed in all farms affected and an epidemiological investigation were performed.

# Characterization of a novel infectious pancreatic necrosis virus (IPNV) genogroup 6 identified in lake Vänern, Sweden

# David B. Persson<sup>1</sup>, Jacob Günter Schmidt<sup>2</sup>, Arianna Comin<sup>1</sup>, Mikael Leijon<sup>1</sup>, Mikhayil Hakhverdyan<sup>1</sup>, Niels-Jørgen Olesen<sup>2</sup> and Charlotte Axen<sup>1</sup>

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#### Abstract

Infectious pancreatic necrosis virus (IPNV) is an important viral pathogen in farmed Atlantic salmon and rainbow trout and seven different genogroups have been described. In Europe, genogroup 5 (previously serotype Sp) is the one causing most severe disease and genogroup 2 (previously serotype Ab) is considered relatively harmless. The Swedish inland zone is considered free from IPNV as well as other viruses such as viral haemorrhagic septicaemia virus (VHSV) and infectious haematopoietic necrosis virus (IHNV). Vertical transmission occurs at least with IPNV and also with Renibacterium salmoninarum causing bacterial kidney disease (BKD), that is present in some Swedish inland waters and in the Baltic Sea. Thus, Sweden has a control program for all wild salmonid restocking farms for prevention of vertical transmissions. In December 2016, IPNV genogroup 6 was detected in a trout from the restocking facility in Sweden's largest lake (Vänern). The farm restocks two salmon (Salmo salar) and two seatrout/brown trout (S. trutta) strains. These strains have been landlocked in lake Vänern since the last ice age. Little is known about IPNV genogroup 6. The eggs of the infected female were destroyed and thus the potential pathogenic effect of the virus on the offspring could not be determined. Instead, an experimental trial was performed at DTU in 2019. Three fish species were used: rainbow trout (Oncorhynchus mykiss, provided by DTU) and two of the Vänern strains that are potentially naturally exposed to the virus: Klarälven salmon and Gullspångsälven trout.

Experimental infection in approximately 1 g offspring from Klarälven salmon, Gullspångsälven trout and control rainbow trout was performed in accordance with the current animal welfare regulations (Directive 2010/63/EU) and were approved by the Danish Animal Research Authority under the license 2013-15-2934-00976. Negative control groups and the IPNV genogroup 5 isolate 16885-2 Rindsholm were used for comparison. The infection dose for both IPNV isolates was set to  $1.3 \times 10^8$  virus particles ( $2.6 \times 10^5$  TCID50/ml water) and infection made through immersion 6 h in 4 L water. All regimens were run in duplicates, i.e. two tanks of negative controls (NC), Rindsholm (R) and Swedish IPNV (Sw) per fish species (N= 100 fish/tank). The fish were monitored daily for 33 days p.i. and disease signs or mortality were logged. Moribund and dead fish were collected and sampled for viral culture. In addition, standardized samplings were performed day 12 p.i. (N=5 fish per tank) and at the end of the trial (day 34 p.i. (N=10 NC, 5 R and 5 Sw fish per species)).

The salmon was least sensitive to infection with both isolates. The Swedish IPNV genogroup 6 isolate caused less infection than the IPNV genogroup 5 isolate used. In rainbow trout, the mortality was also significantly lower in the Swedish IPNV isolate group than in the IPNV genogroup 5 group, whereas the mortality in salmon and trout couldn't be completely evaluated. In conclusion the IPNV genogroup 6 apparently pose a minor to negligible risk to the indigenous salmon and trout species.

## Health status of farmed cyprinids in the Czech Republic Pojezdal L<sup>1</sup>, Mikulaskova K<sup>2</sup>, Motlova J<sup>1</sup>, Matejickova K<sup>1</sup>, Minarova H<sup>1,3</sup>, Palikova M<sup>3</sup>, Piackova V<sup>4</sup>.

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## Abstract

In the landlocked Czech Republic, cyprinids represent the most important aquaculture animals, reared in almost 41 000 ha of interconnected shallow ponds of varying sizes. With a stable yearly production of around 20 000 tones, the Common carp and herbivore cyprinids represent nearly 90 % of Czech aquaculture production.

The presence of koi herpesvirus (*Cyprinid herpesvirus-3*, KHV) in the country is established since at least 2009 in the form of several outbreak sites per year, with a peak in 2019 (11 outbreak sites, 16 ponds). Conventional and molecular analysis of aforementioned outbreaks confirmed the transport of live fish and the use of non-disinfected equipment as a way of virus dissemination through a specific geographic region. In the year 2021, six KHV positive sites were detected, mostly via reported clinical signs and mass mortality events. Active surveillance of the disease, with around 100 sampled sites per year, did not detect any latent KHV infections. On the contrary, two asymptomatic sites were positive for the Carp edema virus (CEV), along with additional six locations sampled from clinically diseased animals.

No cases of spring viraemia of carp (SVC) or carp pox (CyHV-1) were reported in 2021, although the viruses are present in the aquaculture, with latest confirmed isolations in the year 2020.

Despite the fish being considered an invasive species, several reports of specific mortalities of the Prussian carp (*Carassus gibelio*) were recorded, with at least one case laboratory confirmed as CyHV-2 infection.

This study was supported by the Ministry of Agriculture of the Czech Republic MZE-RO0518 and NAZV QK1710114; and the project PROFISH CZ.02.1.01/0.0/0.0/16\_019/0000869 financed by ERDF in the operational program VVV MŠMT.

SESSION III Results from ongoing research on listed and emerging fish diseases Chair : Morten Schiøtt

21

# Prevention of PD in Atlantic salmon by vaccination – experience from field Britt Bang Jensen, Katherine R. Dean, Victor H.S. Oliveira

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## Abstract

In Norway, 140-170 marine aquaculture farms each year are infected with salmonid alphavirus (SAV). In many cases, infection leads to outbreaks of Pancreas Disease (PD), causing mortality and reduced growth in salmonid fish kept for food production. Producers have widely used PD vaccines since 2009, with little effect on the number of outbreaks. These vaccines are based on inactivated SAV. In 2018, a new DNA-based vaccine was licensed. In 2020, this vaccine accounted for 50% of the PD-vaccines sold.

In 2021, we observed near 40% reduction in the number of PD-cases from 158 the previous year, to 101. Thus, it is speculated that the decline in cases is due to the new vaccine.

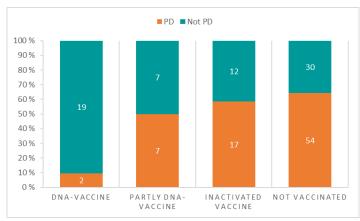
In order to investigate this, a small survey was carried out in two seperate geographic areas of Norway: One where SAV3 is endemic, and one where SAV2 is endemic. We sent out questionnaires asking about the number of fish vaccinated against PD, and what vaccine was used to farmers in these two areas. Farms that had stocked fish in 2018-2021 and where the production had been finished by the end of 2021 were included. In total, 42 farms in one area and 59 farms in the other responded, which constitutes 32% and 48% of the farms, respectively.

The data on use of vaccination was compared with official data on the occurrence of PD from a screening program for SAV that covers all of Norway.

The results showed that all fish in the SAV3-endemic area were vaccinated against PD. In this area, 40% of the fish groups had PD. In the SAV2-endemic area, 12% of the fish were vaccinated. In this area, 64% of the fish groups had PD.

The size of the dataset is not large enough to allow for statistical analysis, but the results nevertheless indicate that the use of PD-vaccination seemed to have had some effect in decreasing the number of PD-outbreaks.

While most fish stocked in 2018 were vaccinated with inactivated PD-virus, in 2020, the majority of fish were vaccinated with the DNA-vaccine. Figure 1 shows the number of fishgroups that got PD, based on the vaccine status.



# Effect of Water Temperature on the Infection Kinetics of *Piscine orthoreovirus* Genotype 3 in Rainbow Trout

Juliane Sørensen<sup>1</sup>, Niccolò Vendramin<sup>1</sup>, Argelia Cuenca<sup>1</sup>, Anne Berit Olsen<sup>2</sup>,

Kerstin Skovgaard<sup>3</sup>, Niels Jørgen Olesen<sup>1</sup>.

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## Abstract

*Piscine orthoreovirus* genotype 3 subtype b (PRV-3b) is widespread in Denmark, but is only associated with disease outbreaks with increased mortality on some occasions. Importantly, disease outbreaks associated with PRV-3 have only been observed in recirculating aquaculture systems (RAS), and have predominantly been observed during the winter.

To elucidate the potential effect of water temperature on the infection kinetics of PRV-3 in rainbow trout (*Oncorhynchus mykiss*), an experimental *in vivo* cohabitation trial was conducted at three different temperatures: 5°C, 12°C, and 18°C. For each temperature, there was one control group (mock-injected with diluted naïve blood) and one PRV-3 exposed group (i.p. injected with diluted PRV-3 positive blood) with a 50:50 ratio of shedders and cohabitants. The experimental trial lasted for 12 weeks, with samplings every second week post challenge (WPC). The fish were not acclimatised to the respective temperatures prior to challenge.

In heart tissue of cohabitants, the viral load of PRV-3 was at the highest level at 6 WPC for both 12°C and 18°C, while viral load peaked at 12 WPC at 5°C. Notably, only 16 fish out of 36 were tested positive for PRV-3 during the experimental trial at 5°C and 18°C compared to 27 fish at 12°C.

In the heart tissue of shedders, the level of PRV-3 peaked at 2, 4, and 6 WPC at 18°C, 12°C and 5°C, respectively. At 18°C and 12°C, the viral load decreased steadily thereafter. Interestingly, the shedders at 5°C did not seem capable of clearing the infection, as virus load remained high until the end of the trial.

Typically, a decrease in the haematocrit level is observed in response to high virus levels. This holds true for both 5°C and 12°C, in which the cohabitants experience a decreased haematocrit level at 12 and 6 WPC, respectively. No change was observed in the cohabitants at 18°C.

Histopathological changes in the heart are usually observed as a consequence of the inflammation triggered by infection with PRV-3. At 12°C in the cohabitants, high histoscores were observed 2 WPC following high virus load. No changes were observed in the hearts of cohabitants at either 5°C or 18°C.

In the shedders, it seems that there was a delay in the development of lesions in the heart at 5°C compared to 12°C; lesions were observed 2 WPC after high virus load at 12°C, but not until 4 WPC after high virus levels was reached at 5°C.

Preliminary data of immune gene expression of the cohabitants on spleen tissue point towards an altered expression profile at 5°C.

# First detection of Cyclopterus lumpus virus (CLuV) in England, following a mortality event

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#### and Stone DM

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#### Abstract

Lumpfish (*Cyclopterus lumpus*) are used as cleaner fish to reduce lice burden on commercial Atlantic salmon and are translocated internationally for this purpose. In September 2021 a site in Dorset, England experienced significant increased mortalities in consignments of Lumpfish hatched and ongrown from eyed ova imported from Norway. The company veterinarian suspected the population to be expressing signs of infection with lumpfish flavivirus (CLuV) and commercial testing provided presumptive confirmation of a CLuV infection using reverse-transcription quantitative polymerase chain reaction (RT-qPCR). Affected fish were subsequently resampled for virus isolation in cell culture, bacteriological and histopathological analysis, as well as real-time and conventional PCR amplification for multiple pathogens as part of a full disease screen. Published sequence data for CLuV was used to design a conventional PCR assay to enable confirmation by sequence analysis. Samples tested from 4 life stages across the site were positive by qPCR for CLuV (Ct values between 19 and 29), and sequence analysis of a 568bp conventional PCR amplicon showed 99.63% nucleotide identity with the published sequence for CLuV. To our knowledge this is the first report of CLuV in England and as this disease has only relatively recently emerged in aquatic animals its likely origin, distribution and impact is largely unknown.

# Overview of the OIE Collaborating Centre for Emerging Aquatic Animal Diseases Richard Paley

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### Abstract

It is well known that global aquaculture production has increased and diversified rapidly in recent decades and has surpassed capture fisheries as a source of aquatic animal protein. This trend is set to continue, with the requirement estimated to be a doubling of production to meet global need by 2050 (FAO, 2020). A major constraint in achieving this goal are aquatic animal diseases in aquaculture sectors globally. An **emerging disease** is defined as a new infection resulting from the evolution or change of an existing pathogen or parasite resulting in a change of host range, vector, pathogenicity or strain; or the occurrence of a previously unrecognized infection or disease. To mitigate the effects of these diseases it is critical to achieve rapid detection and characterisation of the causative agent(s), develop accurate diagnostic tests, understand their epidemiology, and to disseminate the information efficiently to raise awareness to facilitate control measures.

The OIE Collaborating Centre for Emerging Aquatic Animal Disease (CCEAAD) is based at the Cefas Weymouth Laboratory and heads a network of laboratories residing in major aquaculture producing regions globally. A key objective of this network is to harmonise and exchange information and expertise to improve disease surveillance globally. Here we provide an overview of the CCEAAD and report on recent work, aims and aspirations.

SESSION IV:Update from the EURL

# **EURL Training Courses for 2022**

#### Niccoló Vendramin and Tine Moesgaard Iburg

EURL Fish Diseases, National Institute of Aquatic Resources, Technical University of Denmark, Denmark <u>niven@aqua.dtu.dk</u> and <u>timi@aqua.dtu.dk</u>

### Abstract

In 2020 and 2021, due to Covid-19 pandemics the EURL for fish diseases has organized training course virtually.

In 2022, while keeping updated with the development of the pandemic, we aim at having two physical courses

# In week 41 - Methods for implementation of surveillance procedures for listed fish diseases

# In week 42 – introduction to histopathology for fish and crustacean disaeses

Plans and ideas for 2021 will be discussed with the floors.

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

More information are available on the EURL website

www.eurl-fish.eu

## **2022 Inter-laboratory proficiency test for fish diseases** Niccoló Vendramin, Teena Vendel Klinge and Niels Jørgen Olesen

EURL for Fish and Crustacean Diseases,

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#### Abstract

A comparative test of diagnostic procedures. InterLaboratory Proficiency Test (ILPT) 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 causing the notifiable diseases: viral haemorrhagic septicaemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV), and epizootic haematopoietic necrosis virus (EHNV) or related rana-viruses and in addition the fish pathogenic viruses: other fish rhabdoviruses as pike fry rhabdovirus (PFR),spring viraemia of carp virus (SVCV) and infectious pancreatic necrosis virus (IPNV) by cell culture based methods. PT2 was designed for 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). As in previous years, Salmonid Alphavirus (SAV) was included in the panel of pathogens to be investigated should include. Since SAV is not a listed disease in the European legislation, testing for SAV was done on voluntarily base. The EURL would then take care of calculating the score accordingly.

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.

The presentation will focus on highlights of the ILPT for 2022. A specific online meeting discussing all details on report was held in april with all participants.

# EURL for Fish Diseases, work done in 2021 Niels Jørgen Olesen and Niccolò Vendramin

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## Abstract

The duties of the EURL are described in the REGULATION (EU) 2017/625 (OCR). The duties mainly concern the fish cat A, C and E diseases given in (EU) 2018/1882 : Epizootic haematopoietic necrosis (EHN), Infectious salmon anaemia (ISA), viral haemorrhagic septicaemia (VHS), infectious hematopoietic necrosis (IHN), and koi herpes virus disease (KHVD).

The facilities supporting the activities of the EURL are placed in the DTU Campus in Kgs. Lyngby, and placed in the institute DTU AQUA, National Institute of Aquatic Resources.

Due to Covid-19 pandemic and related travelling restrictions, most meeting activities had to be converted to on-line events, whereas the activities that required travelling were cancelled.

The 25th Annual Workshop of the National Reference Laboratories for Fish Diseases was held virtually, using the zoom platform, on 31<sup>st</sup> of May and 1<sup>st</sup> of June 2021. The virtual organization of the meeting has allowed a significant expansion of the number of participants attending the workshop as well as the number of oversea countries participating. The number of participants has reached 103 participants from 45 countries attending over the two days period. There were four sessions with a total of 14 presentations. On May 31st, a workshop on the new Animal Health Law (AHL) was organised. This session was attended only by the staff of the National Reference Laboratories in EU and EFTA countries. The aim of this session was to introduce the new legislative framework which was adopted on 21st of April 2021, and to provide an overview of the methods used for diagnostics of the diseases listed in the new AHL

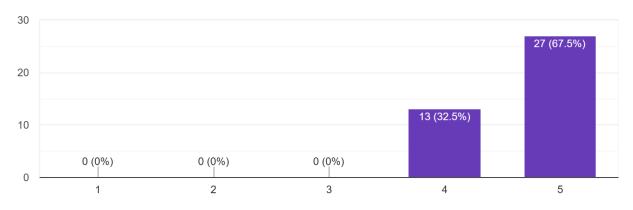
The annual proficiency test for fish diseases (PT) was divided into PT1 and PT2 with 45 laboratories participating. The tests were sent from the EURL 2th of October 2021. The full report with the results and the identification of NRL has been submitted to the Commission, whereas each participant has received: 1- Coded version the report, 2- Certificate of performances indicating also the laboratory code, and if underperformances were observed, a comment explaining potential reasons for this and 3- An email with comments on sequencing and genotyping results .

An important focus of the EURL is to update the standard operating procedures of the non-exotic and exotic listed diseases. In 2021 the EURL has focused on improving the diagnostic manual for IHN and VHS.

During 2021, 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 supply reference materials to NRLs; to provide training courses in laboratory diagnosis; to update the EURL website (www.eurl-fish.eu), to provide consultancy to NRL's and finally to attend international meetings and conferences.

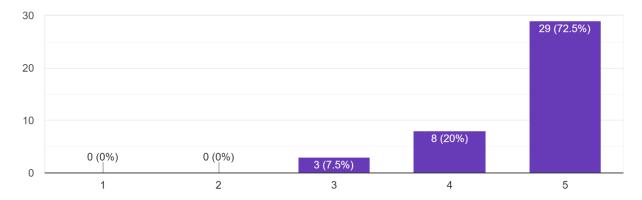
# Workshop evaluation

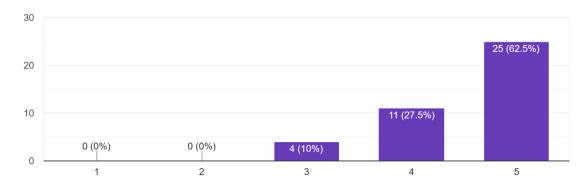
A questionnaire was delivered to the participants asking to evaluate various aspect of the workshop. An overview of the 40 questionnaires retrieved is shown below.



Session I: Update on important fish diseases and their control - Quality of presentations 40 responses

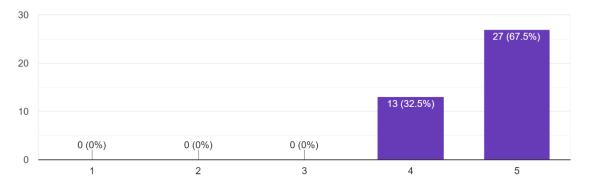
Session I: Update on important fish diseases and their control - relevance for you 40 responses





Session I: Update on important fish diseases and their control - increase of your knowledge <sup>40 responses</sup>

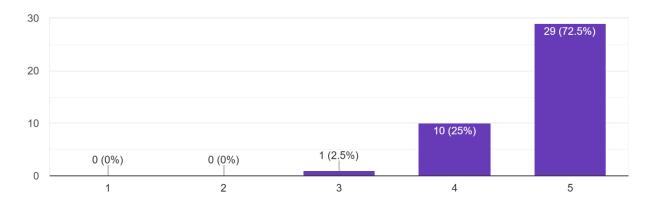
Session I: Update on important fish diseases and their control - overall score <sup>40</sup> responses



Session I: Update on important fish diseases and their control - comments, remarks, inputs

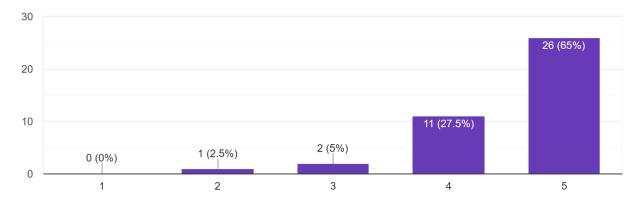
# 7 responses

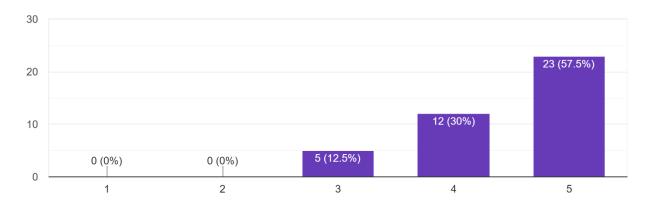
- 1. generally, for all sessions, it might help if the organizers ask the presenters to speak loud and clearly or provide microphones; some presentations were hard to follow
- 2. great satisfaction and use of new information in the future
- 3. Very bad audio
- 4. Well done
- 5. Of course the IT-problems caused distraction from the content. Also, wifi was stopped very frequently for my account, and this was giving disturbance. This last point lasted the 3 days.
- 6. Talks were thoroughly enjoyable and informative. I was hoping there would be an update on RSD. Would be nice to have this next year, although I do understand that it is not a listed disease and preference is needed for those.
- 7. Excellent



SESSION II: Control and Surveillance of fish diseases in Europe- Quality of the presentations 40 responses

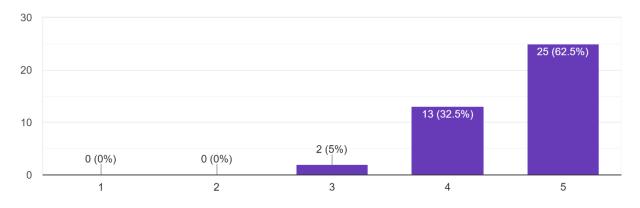
SESSION II: Control and Surveillance of fish diseases in Europe- relevance for you 40 responses





SESSION II: Control and Surveillance of fish diseases in Europe- increase of your knowledge 40 responses

# SESSION II: Control and Surveillance of fish diseases in Europe- overall score 40 responses



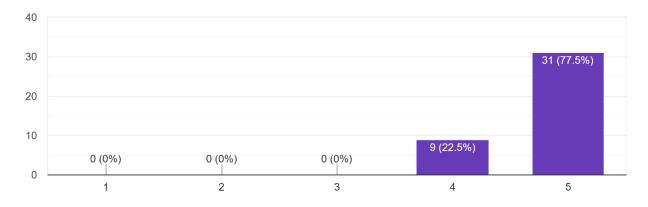
SESSION II: Control and Surveillance of fish diseases in Europe- comments, remarks inputs

# 5 responses

- 1. great satisfaction and use of new information in the future
- 2. bad microphone
- 3. Everything is good
- 4. Very interesting on the "new" diseases Richard Paley and Lenaïg Louboutin reported on.
- 5. As above.

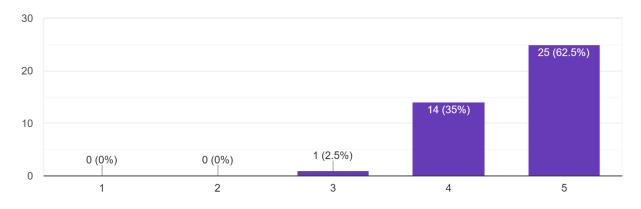
SESSION III: Results from ongoing research on listed and emerging fish diseases-quality of the presentations

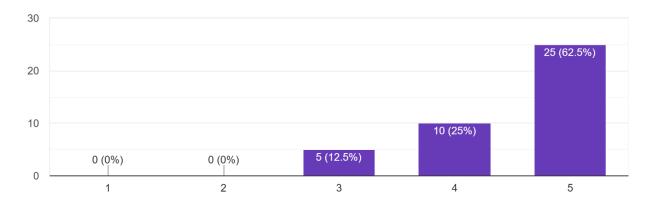
40 responses



SESSION III: Results from ongoing research on listed and emerging fish diseases-increase of your knowledge

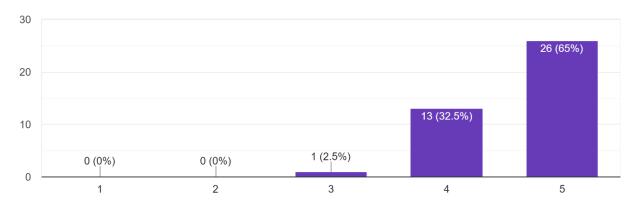
40 responses





SESSION III: Results from ongoing research on listed and emerging fish diseases-relevance for you <sup>40 responses</sup>

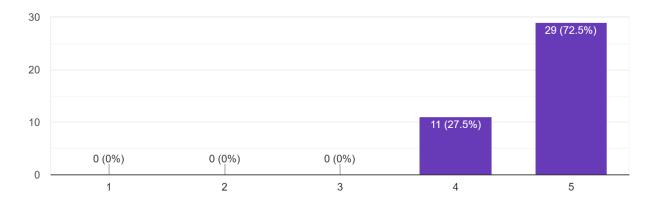
SESSION III: Results from ongoing research on listed and emerging fish diseases-overall score 40 responses



SESSION III: Results from ongoing research on listed and emerging fish diseases- comments, inputs, remarks

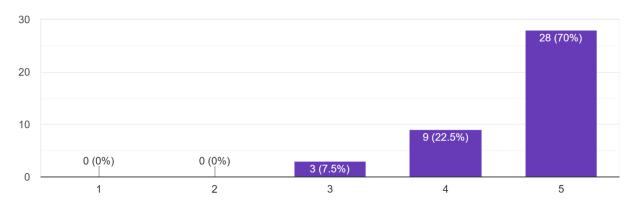
4 responses

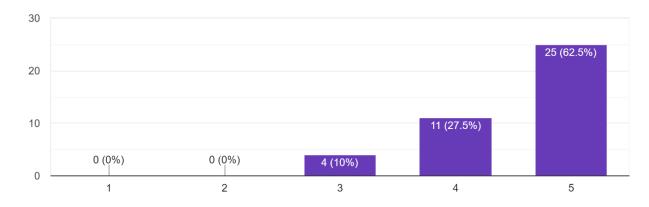
- 1. great satisfaction and use of new information in the future
- 2. Perfect
- 3. Very good!
- 4. Very informative. I would love to see more on cleaner fish as we move forward to get a good idea of what other countries are seeing.



SESSION IV: Update from the EURL for fish diseases- quality of the presentations <sup>40</sup> responses

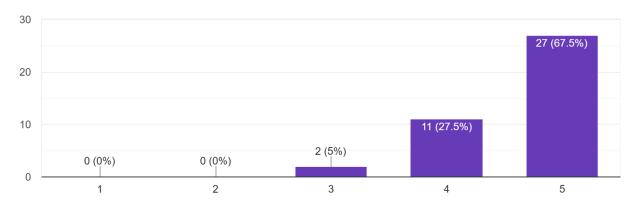
SESSION IV: Update from the EURL for fish diseases- relevance for you <sup>40</sup> responses





SESSION IV: Update from the EURL for fish diseases- increase of your knowledge 40 responses

# SESSION IV: Update from the EURL for fish diseases- overall score 40 responses



SESSION IV: Update from the EURL for fish diseases- comments, inputs, remarks

5 responses

- 1. great satisfaction and use of new information in the future
- 2. Excellent
- 3. Very good, the evaluation of the ILPT of Fish Virology was prepared so well by asking the labs to send discussion points prior to the meeting! I hope we get the PDFs of the lectures of the three days? Thank you! And the meeting was all well organized overall, also regarding the breaks and dinner etc. thank you all so much!!! A big 5 for that! Cheers, Olga
- 4. for the next meeting, please do it physically (not hybrid meeting) so we will be forced ( and it will better accepted by our administration) to come to DK

5. Delighted to hear you will be providing some additional data/instructions for genotyping in the PT

# Greetings and conclusions of the meeting

The next meeting will be held in May 2023 and we aim at having a face to face meeting. Thanks a lot to the people arranging the meeting as well as those of you who helped running the meeting by being chair, presenter and/or participant.

We are looking forward to seeing you all next year!

With kind regards,

The EURL fish and crustacean team