European Union Reference Laboratory for Fish and Crustacean Diseases

NATIONAL INSTITUTE OF AQUATIC RESOURCES, TECHNICAL UNIVERSITY OF DENMARK

Report of the

27th Annual Workshop of the National Reference Laboratories for Fish Diseases

Kgs. Lyngby, Denmark

30th and 31st of May 2023

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

The 27th Annual Workshop of the National Reference Laboratories for Fish Diseases was held on May 30th and 31st 2023.

The number of participants reached 59 participants from 29 countries attending over the two days period. There were four sessions with a total of 24 presentations.

The workshop was held back-to-back with the 14th 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 Section for fish and shellfish diseases, Britt Bang Jensen. 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 2022 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 https://www.eurl-fish-crustacean.eu/ .
Secondly, the fish disease situation in Norway was presented; a detailed report is available at https://www.vetinst.no/rapporter-og-publikasjoner/rapporter/2023/fiskehelserapporten-2022 .

The session continued with two presentations covering relevant topics in the field of fish diseases, covering CEV infection in carps in France (Marine Baud, NRL of France), studies looking at vertical transmission of PRV-3 in rainbow trout in Denmark (Niccoló Vendramin EURL). After lunch break the session resumed with a presentation on Red Skin Disease in wild salmon (Charlotte Axen, NRL of Sweden) and health challenges for farmed Atlantic salmon in Faroe Island (Debes Christiansen, NRL Faroe Island).

After a short coffee break, session II Control and Surveillance of fish diseases in Europe.
This session was planned for nine talks, unfortunately one of the presentations was cancelled due to Covid.

The first presentation given by the representative of the Norwegian NRL Torfinn Moldal, focused on tracing ISAV HPR0 infection hatcheries.
Afterwards, the representative of the Swiss NRL, Heike Schmidt-Posthaus, gave a presentation on Perch Rhabdovirus in Switzerland.
An update on Red Mark Syndrome and its control was given by Jacob Schmidt, from NRL of Denmark.
Later on, a series of 3 presentation targeted IHN. At first, Tine Iburg from Danish NRL, presented a status on the epidemics of IHN in Denmark, then Drazen Oraic from Croatian NRL presented a review of the outbreaks of IHN and VHS occurred in Croatia since 2012. Finally, Argelia Cuenca from EURL for fish diseases presented an overview of molecular characterization of different IHNV isolates causing disease outbreaks in Europe in the past years, encompassing cases occurred in Denmark, Croatia, Finland and Republic of North Macedonia. A fourth presentation on the IHN outbreak occurred in Finland was initially scheduled, unfortunately this was cancelled as the speaker

from Finland got Covid the day before the initiation of the workshop and therefore cancelled her participation.

The session finished with two presentations, one given from Olga Haenen from the dutch NRL, on first detection of PRV-3 in farmed rainbow trout in the Netherlands. Finally, researcher Dr. Lubomir Pojezdal gave a talk on survival to IHNV and VHSV infection in different strains of Rainbow trout. The first day of the meeting was then finalized, participants were transferred then to Bakken for social dinner.

The second day started with Session III, presenting results from ongoing research on listed and emerging fish diseases.
The first presentation was given by Prof. Niels Lorenzen, elucidating antigenic features of Carpione Rhabdovirus.
Then Senior Scientist Britt Bang Jensen presented an assessment of the economic impact of IHN in Danish fish farm.
Later on, a shared presentation between experts from the Italian NRL on the effect of temperature on the replication of different strains of IHNV in vitro, and their virulence in vivo.
Later on, Phd student at DTU Aqua, Alejandra Alonso, presented the results of an experimental challenge trial with *R.salmoninarum* in rainbow trout.
The session finished with a presentation of researcher Dr. Lubomir Pojezdal on immune response to KHV in common carp and Koi carp.

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

In this session the EURL the training course scheduled for October 2023 were advertised. A resume of the InterLaboratory Proficiency test 2022 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 2022 were presented. Furthermore, the EURL activities in year 2022 were presented and proposals for the EURL work plan for 2023 and 2024 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, Niccolò Vendramin and Morten Schiøtt. Niccolò Vendramin assembled the report.
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 was organized by a team consisting of Morten Schiøtt and Niccoló Vendramin, 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 planned to be held online at end of May 2024. More details will follow.

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

Niccolò Vendramin and Britt Bang Jensen

Programme

Tuesday May 30th
Annual Workshop of the National Reference Laboratories for Fish Diseases

10:30 – 10:45 Welcome and announcements
Britt Bang Jensen

SESSION I: Update on important fish diseases and their control

Chair: Britt Bang Jensen and minutes: Morten Schiøtt

10:45 – 11:15 Overview of the disease situation in Europe
Niccoló Vendramin

11:15 – 11:35 Overview of the disease situation in Norway
Torfinn Moldal

11:35 – 11:55 CEV epidemiology in France
Marine Baud

11:55 – 12:15 Viral pathogens fluctuations from broodstock to progeny in rainbow trout with focus on PRV-3
Niccoló Vendramin

12:15 – 13:00 ***LUNCH***

13:00 – 13:20 RSD in wild salmon – current knowledge and future perspectives
Charlotte Axen

13:20 – 13:40 Health challenges for A.salmon farmed in Faroe Island
Debes Christiansen

13:40 – 14:10 ***Coffee break***

SESSION II Control and Surveillance of fish diseases in Europe

Chair: Niccoló Vendramin and minutes: Argelia Cuenca

14:10 – 14:25 ISAV HPRO in hatcheries – detection in different matrixes and possible measures
Torfinn Moldal

14:25 – 14:45 Perch Rhabdovirus (PRV) – Situation in Switzerland
Heike Schmidt-Posthaus

14:45 – 15:05 Control and mitigation of Red Mark Syndrome in Rainbow trout
Jacob Schmidt

15:05 – 15:20 Update on IHN in Denmark
Tine Iburg

15:20 – 15:40 Follow up on IHN outbreak in Finland and its management
Tuija Kantala

15:40 – 16:00 Overview of IHN and VHS outbreaks in Croatia and their management since 2005
Dražen Oraić

16:00 – 16:30 Molecular characterization of IHNV in Europe
Argelia Cuenca

16:30 – 16:50 First detection of PRV-3 in the Netherlands at a rainbow trout farm, July 2022
Olga Haenen

16:55 – 17:15 Survival to IHNV and VHSV challenge of different strains of rainbow trout
Lubomir Pojezdal

Bus transfer to Bakken

Wednesday May 31st
27th Annual Workshop of the National Reference Laboratories for Fish Diseases

SESSION III Results from ongoing research on listed and emerging fish diseases

Chair: Morten Schiøtt and minutes: Niccoló Vendramin

9:30 – 9:40 Welcome and announcements

9:40 – 10:00 Mab IP5B11 binds Carpione Rhabdovirus
Niels Lorenzen

10:00 – 10:20 Assessment of economic impact of IHN outbreak on Danish fish farm
Britt Bang Jensen

10:20 – 10:40 Temperature efficiency of IHNV replication in vitro and vivo
Anna Toffan

10:40 – 11:00 Experimental infection of Rainbow trout with *R.salmoninarum* the causative agent of BKD.
Alejandra Alonso

11:00 – 11:20 ***Coffee break***

11:20 – 11:40 Immune response to KHV infection in common and Koi carp from gene expression to proteomics
Lubomir Pojezdal

SESSION IV Update from the EURL for fish diseases

11:45 – 12:00 EURL Training Courses. Topics and organization of courses 2023
Niccoló Vendramin and Tine Moesgaard Iburg

12: 00 – 12:15 Interlaboratory Proficiency test for fish diseases 2022
Teena Vendel Klinge and Niccoló Vendramin

12:15 – 12:40 EURL Work done in 2023, plan for 2024
Niccoló Vendramin and Britt Bang Jensen

Next meeting and end of 27th Annual Workshop
Niccoló Vendramin and Britt Bang Jensen

END OF FISH WORKSHOP

SESSION I: Update on important fish diseases and their control

Chair: *Britt Bang Jensen*

Overview of the fish diseases situation in europe in 2022

Niccolò Vendramin

[1]*DTU Aqua National Institute of Aquatic Resources, Kemitorvet 202, Kgs. Lyngby,*

niven@aqua.dtu.dk

Abstract

The questionnaire on Survey and Diagnosis of the listed fish diseases in Europe (S&D) for 2022 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 (www.eurl-fish-crustacean.eu) , 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.
2. 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.
3. Laboratory data from the NRLs and other laboratories, including the numbers of samples examined, and diagnoses of fish diseases made.
4. A National report describing health and surveillance situation in general. These reports are compiled into one and can be found on https://www.eurl-fish-crustacean.eu/ .

Production data for 2021

The most update data on aquaculture production in Europe refer to 2021 on the website of Federation of European Aquaculture producers (draft produced by FEAP secretariat on march 2023). We decided to refer to the dataset provided by FEAP .

At global level, the pandemic affected 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.

A recent report from EUMOFA (2023) has also investigated high ends aquaculture product in the European Union (i.e. Turbot and Caviar) and reported a significant impact on trade of these two commodities especially during the initial impact of the pandemic.

The total fish production in aquaculture in Europe, including Turkey and Norway, increased slightly from 2020 and is now at 2,875,732t. The total production of EU countries has increased in 2021 from 505,734 tonns to 537,197.

Number of fish farms in Europe

The total number of authorised/licensed fish farms in Europe is estimated in about **35514** farms. This estimates suffer three type of bias:

1- Four participants did not provide a reply
2- In some instances put and take lakes are counted as farms

3- The figures received do not state whether a farm is active or not

Germany provide the largest contribution when it comes to fish farm with 14626 farms, while the second contributor is Poland with 5191. In both cases, this high nubmer of farms reflect a large number of small size farms.
When it comes to production, Norway has by far the largest production in Europe and has licensed 1326 farms/sites.

Health status of fish farms

There are currently four possible health statuses:

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

In 2022, a health status was assigned to 14692 farms with susceptible species to VHS, to 15495 farms with susceptible species to IHN, to 5004 farms with susceptible species to ISA.

Health status for VHS, 16% of fish farms are approved disease free; 2% is under eradication/control program; 11% under voluntary program; 72% is not approved disease free and not under eradication/control program

Health status for IHN, 21% of fish farms are approved disease free; 1% is under eradication/control program; 11% under voluntary program; 67% is not approved disease free and not under eradication/control program

Health status for ISA (Infection with HPRΔ ISAV), 66% of fish farms are approved disease free, 0% is under eradication/control program, 1% under voluntary program, 33% 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**, 13 new outbreaks were reported in Europe in 2022, 6 of these were in Germany. The remaining outbreaks were observed in Switzerland (1), Romania (3), Poland (1), Austria (2).

For **IHN**, 80 new outbreaks were reported. The majority was in the Republic of North Macedonia (30), followed by Denmark (19). The remaining outbreaks were in Germany (16), France (2), Finland (1), Italy (1), the Netherlands (6), Poland (2) and Slovenia (3). A series of 4 presentation will focus on IHN situation during 27th Annual workshop for National Reference Laboratories for Fish Diseases.

For **ISA** (Infection HPRΔ ISAV) Norway reported an decreased number of outbreaks compared to 2021 (20). A specific talks on the topic will be provided at the 26th Annual Workshop for National Reference Laboratories for Fish Diseases.

For **KHVD**, 48 outbreaks were reported in 2022.

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), Enteric Redmouth Disease (*Y. ruckeri*) with a concern for biotype 2, Furunculosis (*A. salmonicida*), Bacterial kidney disease (BKD), and Infectious Pancreatic Necrosis. An outbreak of co-infection with PRV-3 and Fl.psychrophilum was reported in the Netherlands.
- 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 and parasitic infestations with ectoparasites including *Ichthyophthirius multifiliis*
- In **seabass** and **seabream** it is primarily VNN/VER, *Photobacterium damselae* subsp. *Piscicida*, tenacibaculosis, *Vibrio harvey*, *Sparicotyle chrysophrii* infection, Piscirickessia salmonis is also reported.

Q:CMS HSMI problem with case definition

A: diagnosis: detection of virus presence of heart lesion, difficult to define an outbreak - how many fish should show signs? No rules. No true data to verify of incidents are going up or down.

Q: Presence of virus does not necessarily lead to clinical signs.

Comment : difficult to compare between countries as definitions are different

Overview of the disease situation in Norway

Torfinn Moldal, **Ingunn Sommerset, Jannicke Wiik-Nielsen,**

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

Norwegian Veterinary Institute

torfinn.moldal@vetinst.no

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 2022 in Norwegian was published in March, and the English version is expected to be published in late May on https://www.vetinst.no/rapporter-og-publikasjoner/rapporter.

The main species in Norwegian aquaculture is Atlantic salmon. In 2022, more than 56 million farmed salmon died during the seawater phase of the production. The probability of a fish dying during the year was 16.1% on national level. However, there is great variation between the thirteen production areas with highest probability in the western part of the country. The main reasons for mortality are reported by fish health personnel to be mechanical injuries after delousing operations, CMS, infections caused by *Moritella viscosa* and *Tenacibaculum* spp., complex gill diseases and HSMI.

Among the notifiable viral diseases, infectious salmon anemia (ISA) was confirmed at 15 sites in 2022. In addition, ISA was suspected at five sites based on detection of virulent ISA-virus. ISA was confirmed or suspected at one or more sites in every production area (PA), from PA2 in the southwest to PA10 in the north. There was at least three cases where ISA virus likely had been transmitted between neighbouring sites, while two confirmed outbreaks at ongrowing sites could be linked to the smolt farms that had delivered fish to the respective sea sites and two confirmed outbreaks in brood fish farms could be linked to relocation of fish from a third brood fish farm where ISA was suspected.

Pancreas disease (PD) was confirmed at 98 sites in 2022. All detections were within the endemic zones for SAV2 and SAV3, and there were equal numbers of detections of each genotype. It is worth noting that there was a marked increase of detections of SAV2 in PA6 and a pronounced decrease of detections of SAV3 in PA3.

A risk-based surveillance programme for infectious hematopoietic necrosis virus (IHNV) and viral haemorrhagic septicaemia virus (VHSV) based on examination of samples submitted for routine diagnostic investigation is in place. In 2022, brown trout at cultivation and ongrowing sites as well as rainbow trout at inland sites were also included in the surveillance programme. Neither IHNV nor VHSV were detected in Norway in 2022.

Several bacterial infections have effectively been controlled by vaccination for decades. However, winter ulcers were diagnosed at 433 sites with Atlantic salmon in 2022. Pasteurellosis was diagnosed at 52 sea sites with Atlantic salmon in the western part of the country, while yersinosis was diagnosed at 33 sites with farmed Atlantic salmon in different production phases and several parts of the country.

Q: Gyrodactylus, still using rotenon treatment?

A: Yes, but also chlorin

Q: What does companies want to give numbers for (non-)notifiable diseases

A: Mutual Benefits

A: Companies would like to know the reasons for production losses, and for companies for not being blamed for e.g. environmental issues

Q: Any comments from environmental organizations?

A: Not really

Q: Diseases reported directly to NRL in real time?

A: Samples taken by private vets, not reported directly to NRL

Q: QTL resistant fish

A: Diseases also affecting QTL resistant fish

Q: Are the incidents of Nephrocalcinosis from flow through or RAS systems

A: Both in RAS and flow through

Koi sleepy disease in France: Focus on a series of outbreaks in 2022.

Marine Baud[1], Doriana Flores[1], Laurane Pallandre[1], Hélène Giummarra[1], Armand Lautraite[2], Rodolphe Thomas[3], Françoise Pozet[4], Nicolas Keck[5], Amélie Charrier[6], Marine-Océane Guillermard[7], Jésabel Laithier[7], Aurélien Tocqueville[7], Sophie Le Bouquin-Leneveu[3], Laurent Bigarré[1]

[1] *ANSES, Ploufragan-Plouzané-Niort Laboratory, 29280 Plouzané, France*
[2] *Private Veterinary, 82170 Grisolles, France*
[3] *ANSES, Ploufragan-Plouzané-Niort Laboratory, 22440 Ploufragan, France*
[4] *Departmental Laboratory of Jura, 39802 Poligny, France*
[5] *Departmental Laboratory of Hérault, 34030, Montpellier, France*
[6] *Departmental Laboratory of Pyrénées and Landes, 40004 Mont de Marsan, France*
[7] *ITAVI, 75009 Paris, France*

Email of corresponding author lnr.poissons@anses.fr

Abstract

The CEVIRAL project (funded by EMFF) aims to acquire epidemiologic and molecular data on the Carp Edema Virus (CEV) in France. CEV is responsible of the Koi sleepy disease which has spread in France since the 2010's (Flores *et al.*, 2023). Preliminary studies showed the presence of both genotypes I and II in the country, circulating in common carp and Koi, respectively (Baud *et al.*, 2021). From the end of 2020 to May 2023, samples were collected during outbreaks affecting common carps and Koi, in farms or in natural ponds. A questionnaire was completed by stakeholders to collect epidemiological data (origin of fish, mortality, water temperature). Real-time PCR-based (Matras *et al.*, 2017) diagnosis were performed by three departmental laboratories and positives samples sent to ANSES for genotyping.

Hereby, we present an epidemiological study of a series of mortalities observed on common carps in the Tarn 'département' during spring 2022. Four mortality events were reported in four ponds of various sizes, distant from several kilometers and without hydric connection. For three of these episodes, Carp edema virus (CEV) was detected in fish, suggesting that the sleepy disease was the cause of the deaths. One site could not be sampled but the koi sleepy disease was nevertheless suspected based on clinical and epidemiological features. Three episodes were associated to restocking operations with mirror carps several months earlier. Sequencing of the complete P4a gene showed a nearly-complete (100 %) nucleic acid identity between the three sampled viruses, all from genotype I, suggesting the following hypothesis: the virus would have been transmitted to the common carps in each pond as soon as the end of 2021, triggering the disease later on, in 2022. About the 'La Raviège' lake, the last of the four lakes hit by mortalities and the largest one, the introduction of the virus is not linked to a re-stocking initiative. For this site, an epidemiological link with the other sites is suspected, but remains to be elucidated.

References

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Matras, M., Borzym, E., Stone, D., Way, K., Stachnik, M., Maj-Paluch, J., Palusińska, M. and Reichert, M. 2017. Carp edema virus in Polish aquaculture – evidence of significant sequence divergence and a new lineage in common carp Cyprinus carpio (L.). *J Fish Dis* 40, 319-325, doi: 10.1111/jfd.12518.

Q: Was it possible to get sequences from the company providing restocking

A: NRL cannot contact provider, as it should be anonymous

Q: Maybe Koi carps released in the lake can transfer disease?

A: Yes

Q: Water temperature?

A: 10 - 14, March to June in carps, later in Koi carps.

Q: symptoms, do you see clinical signs

A: lots of mucus on fish and gills. No histopathology.

Q: Koi sleepy diseases eyes can go inwards, lying on the bottom.

A: Fish farmers do not care about the disease signs

Q: No CEV in first lake, go back after temp increase?

A: No, it was too late when the results had been produced after 2 - 3 months.

Q: Tested for KHV?

A: Owner does not like to know about KHV.

Viral pathogens fluctuations from broodstock to progeny in rainbow trout with focus on PRV-3

Niccoló Vendramin, Eleni Zagopoulo, Juliane Sørensen, Argelia Cuenca

DTU AQUA, Section For fish and shellfish diseases, EURL for fish and crustacean diseases

niven@aqua.dtu.dk

Abstract

Piscine orthoreovirus (PRV) are double stranded RNA virus which infect salmonids worldwide.

Three different genotypes are currently recognized, PRV-1 causing Heart and Skeletal Muscle Inflammation in Atlantic salmon, PRV-2 associated with Erythrocytic Inclusion Body Syndrme in Coho salmon and PRV-3 which targets primarily rainbow trout.

The genotype 3 (PRV-3) was detected for the first time in Norway and later in Denmark. Earlier studies suggest that the transmission of PRV-3 in Danish farms follows trade of subclinical infected fish; furthermore efficient horizontal transmission has been demonstrated under experimental conditions. Little is known about the potential for vertical associated transmission of PRV-3, in combination with the prevalence of the virus in the larval stage as well as the broodstock.

The screening for PRV-3 has taken place in a hatchery. In the facility, broodstock are maintained in concrete tanks in RAS facility. Once eggs are stripped and fertilized they are hatched in a separate system supplied with borehole water.

The facility has been found positive for PRV-3 in a previous survey and the status for IPNV is "not officially free".

Samples from four time points during production, blood from broodstock, gametes at stripping, larval stage soon after hatching , larval stage at "swim up" phase.

Interestingly, where it is possible to detect PRV-3 in blood from broodstock by qPCR, the virus is not detected in any of the following sampling points. Conversely possible traces of IPNV are detected throughout the production.

Preliminary results will be discussed with a focus on expected prevalence, and pitfalls on test sensitivity and specificity.

Q: High Ct values alternative assay, tested in triplicate? The high Ct values are very uncertain.

A: That will be done. But for PRV-3 the story seems like no vertical transmission has occurred.

RSD in wild salmon – current knowledge and future perspectives

Charlotte Axén, David Persson, Riikka Holopainen[2], Niccolò Vendramin[3], David Persson[1], Marjukka Rask[2], Tine Moesgaard Iburg[3], Hampus Hällbom[1], Satu Viljamaa-Dirks[2], Jacob Schmidt[3], Argelia Cuenca[3]

[1]National Veterinary Institute, Dept for Animal health and antimicrobial strategies, Uppsala, Sweden, [2]Finnish Food Authority, Finland, [3]National Institute of Aquatic Resources, Technical University of Denmark, Kongens Lyngby, Denmark

charlotte.axen@sva.se

Abstract

Fresh run Baltic salmon with skin haemorrhage started to appear in Swedish rivers in 2014. The red skin disease (RSD) was first seen in Mörrumsån (Southern Sweden) in early May. In late June, RSD was seen in Torneälven, the border between Sweden and Finland. RSD has re-occurred in Baltic Sea rivers every year since. A few RSD-affected fish were reported in Swedish west coast rivers 2017-2018 and since 2019, RSD has also been reported from Norway, the UK and Ireland. Skin haemorrhage (acute to healing), fin lesions, secondary saprolegniosis and sometimes UDN-like lesions on the head are seen. Various bacterial and viral diseases have been excluded as the cause of RSD by cultivation or PCR. Histopathology showed an inflammatory response in the dermis and hypodermis even in acute lesions, indicating an underlying inflammatory process rather than a response to the lesions. This picture resembles red mark syndrome (RMS) in rainbow trout. UDN-like lesions are not seen in all rivers, and it is unclear whether there is a link to RSD or not.

In 2020, skin samples were sent to the Technical University of Denmark (DTU) to follow up on the similarity of RSD to RMS. RMS is proposed to be caused by an intracellular bacteria called "Midichloria-like organism" (MLO). Real-time PCR for MLO produced non-specific amplification, but the melting temperature was the same for all RSD samples showing that whatever amplified has the same DNA sequence. The melting temperature differs from MLO, thus there is another organism, probably similar to MLO. More RSD skin samples from 2021 and 2022 samplings in different rivers in Sweden and Finland produced the same results.

In 2023, an application has been sent to AquaExcel3.0, to enable an infectious trial at DTU. The infection trial will be based on experiences from RMS trials. However, it will not be possible to use adult salmon, thus co-habitation with naturally diseased fish is impossible. Salmon (30-50 g) will be used and i.p. injected with homogenate from frozen RSD affected skin, collected from Torneälven and Umeälven in 2023, and observed for 3 months. Due to a shortage of time for planning logistics, it is not possible to perform the trial with fresh skin in 2023. If RSD doesn't develop, a new trial using fresh RSD affected skin will be performed in 2024. If RSD develops, another trial with co-habitation fish is planned. NGS will be performed on affected tissue and used to map the DNA sequence of the organism, whereafter a specific qPCR for the "RSD-MLO" can be developed.

Q: Did you consider primary cause by e.g. mechanical injury by predators, grey seals like atlantic salmon, bite or scratch salmons

A: Fish with symptoms don't always have injuries

Q: have you tried primary cultures of salmon skin? Maybe pathogen will grow in those? Inoculation by scratching the skin may also help.

A: Good idea to try.

Q: Do you collect the samples yourself? How to get information about sick fish

A: 20 random fish from a river examined to also measure disease incidents.

The Fish Health Barometer in Faroese Aquaculture

Debes Hammershaimb Christiansen, Senior Researcher and Head of Department

Faroese Food and Veterinary Authority

Debesc@hfs.fo

Abstract

Atlantic salmon farming accounts for around 50% of the Faroese export value. Since the Faroese ISA epidemic in the beginning of the millennium, the annual production has increased significantly and reached a record high of 95.000 tons in 2021. Although the industry ambitions are continuous growth to supply the increasing global demands, we have seen a stagnation in production of in average 74.000 tons (range 65.000 – 95.000) within the last decade. Furthermore, whereas Faroese salmon production was labelled "best in class" with an average annual mortality post sea transfer of 6.2% from 2005 to 2013 the average mortality has more than doubled within the last decade (range 8.5 – 19.8 %). The major driver for the adverse development of growth in salmon production in the Faroes is salmon lice infestation. Since the first signs of the lice becoming resistant against the very efficient chemotherapeutics in 2008 several alternative methods have been introduced including various mechanical treatments. Today, bath treatment in heated water (mechanical treatment) and cleaner fish are among the main strategies used for controlling sea lice in Faroese marine farms. However, mechanical treatment imposes major fish health and welfare issues. Furthermore, it increases the susceptibility to development of various diseases caused by endemic viral and bacterial pathogens including PMCV, PRV-1, BKD, *Aeromonas salmonocida*, *Moritella viscosa* and *Tenacibaculum*.

Another strategy to circumvent the adverse effect of sea lice treatment and the risk of diseases in marine farms is the production of large smolt in RAS to reduce time at sea. However, pathogens established as house strains such as ISAV, POX and IPNV can increase the risk of diseases in large smolts in RAS.

Here I will give an overview of the most important fish health and welfare challenges in Faroese aquaculture with focus on some of the most important pathogens in RAS and marine farmed salmon in the Faroe Islands.

Q: Salmon lice treatment, how high values can you tolerate

A: It's a balance, a few per salmon don't harm, while the treatment does, but it can easily get out of hand.

Q: benchmark of 10 % mortality, where does this number come from?

A: It is feasible to reach that, as it has been done before

Q: mortality due to handling varies – what could be the reason?

A: Some farmers don't use mechanical delousers, chemical treatment is not as harmful.

SESSION II: Control and Surveillance of fish diseases in EU

Chair: Niccoló Vendramin

Perch rhabdovirus (PRV) – Situation in Switzerland

Heike Schmidt-Posthaus, Nicolas Diserens

Institute for Fish and Wildlife Health, Department of Infectious Diseases and Pathobiology, University of Bern, Bern, Switzerland

heike.schmidt@unibe.ch

Abstract

Perch rhabdovirus (PRV) belongs to the genus Perhabdovirus. The perhabdovirus genome is composed of a linear, negative-sense, single-stranded RNA, which codes for 5 proteins. Differences in the nucleoprotein (N) and phosphoprotein (P) genes are generally used to differentiate genetically close viruses.

PRV can lead to high mortality in European perch (*Perca fluviatilis*) and pikeperch (*Sander lucioperca*), mainly larvae and juveniles. In older fish, typical clinical signs are spiral swimming and, later in the disease course, cachexia due to emaciation. In 2013, the virus was first detected in a Swiss aquaculture facility. Driven by intensive fish trade, different variants of PRV are currently circulating in the European percid aquaculture.

Twenty-two PRV cases were diagnosed in Switzerland since 2018: in aquaculture, 13 cases in pikeperch and seven in European perch; in wild European perch populations two different river catchments were affected.

In all Swiss cases, a conventional RT-PCR targeting a 484bp sequence on the N gene followed by sequencing was performed. Finally, a phylogenetic analysis using the Maximum-Likelihood method with 1000 Bootstrap replicates was conducted.

Three main clusters were observed. The largest cluster consisted of 13 isolates from pikeperch. The second one involved seven isolates from European perch. The last cluster consisted of the two isolates detected by wild European perch and was more closely related to the pikeperch cluster.

The results confirm that the virus spread is strongly supported by animal trade, which has led to the appearance of two main isolate clusters – one in pikeperch and one in European perch. Control mechanisms to prevent further spread of the disease should be implemented.

Q: which is the water temperature of the water?

A: 20-22 degrees

Q: Temperature of the cell culture?

A: Also 22 degrees

Q: can the virus be transmitted to other fish species other than percids?

A: Not that we know. We don't have any information if other fish species can transmit the virus.

Q: Is it possible to discuss the nomenclature of these viruses? PRV for piscine orthoreovirus, and Perch rhabdovirus and Pseudorabies virus? It can be very confusing for fish farmers

Q: perch rhabodovirus from brown trout, how different are they in the phylogeny?

A: We still need to do the direct comparison

ISAV HPR0 in hatcheries – detection in different matrixes and possible measures

<u>Torfinn Moldal</u> and Hilde Sindre

Norwegian Veterinary Institute

torfinn.moldal@vetinst.no

Abstract

Infectious salmon anaemia (ISA) is a serious viral disease in Atlantic salmon (*Salmo salar* L.). Non-pathogenic ISA virus (ISAV HPR0) is considered to be the progenitor for pathogenic ISA virus (ISAV HPRΔ) characterized by a deletion in the hyperpolymorphic region of segment 6. ISA has been detected in two hatcheries in Norway during the last ten years. A surveillance programme for ISAV HPR0 in Norwegian hatcheries has been conducted since 2019. The presence of ISAV HPR0 in several hatcheries has been linked to ISA outbreaks at sea sites.

A project funded by the Norwegian Seafood Research Fund (grant number 901674) on biosecurity measures against ISA in hatcheries seeks to increase the knowledge about the transition from non-pathogenic to pathogenic virus and to establish best practice for biosecurity measures that can reduce the risk of change to and spread of ISAV HPRΔ. Traditionally, tissue samples have been used for the detection of ISA virus, but a study involving ten hatcheries demonstrates that water samples and swabs from the environment, skin and gills are suitable for detecting infection at facility level. This is promising for non-lethal sampling for monitoring infection status. Follow-up of two groups of fish from the parr stadium to sea transfer also confirms that swabs can be a good alternative to tissue samples for virus detection and that infection with ISAV HPR0 is transient. In one of the fish groups, however, the virus was detected again after some time.

One hatchery taking part in the project has apparently succeeded in removal of the virus after an ISA outbreak. The work included dismounting of equipment, washing, disinfection and rebuilding of the unit for disinfection of sea water with ultraviolet light.

Through interviews with key people at the hatcheries involved, we will try to understand challenges and practices and use the information in a descriptive analysis of what hatcheries with extensive challenges with ISAV HPR0 do compared to hatcheries without ISAV HPR0.

Q: when they disinfect the filters do they need to thought them away, or can be repopulated?

A:They need to start from the beginning again.

Q: Does the farm had problems with other pathogens?

A: I think, but I don’t have any specific

Control and mitigation of red mark syndrome in rainbow trout

Jacob G. Schmidt

DTU Aqua, Section for Fish and Shellfish Diseases, Lyngby, Denmark

jacsc@aqua.dtu.dk

Abstract

Red mark syndrome is a skin disease affecting farmed rainbow trout. The disease has been a growing problem in European aquaculture since the turn of the millennium. The disease differs from most other diseases in trout aquaculture in several ways: It causes no or little mortality; clinics (large hyperemic skin lesions) are mainly observed in large fish close to sale/slaughter; it is likely caused by an obligately intracellular bacterium related to *Midichloria mitochondrii*, provisionally termed MLO.

The suspected causative agent of RMS has not been isolated nor cultured, thus making controlled experimental work challenging. Instead, a cohabitation model of disease transfer is used for investigations on RMS. At DTU Aqua we have maintained such a cohabitation disease model of RMS for 7 years for experimental purposes. I will present some of our experiments and observations and put them into a context of possible mitigation measures. These include previously publish work such as the effect of antibiotics (Schmidt *et al*. 2021) and temperature (Orioles *et al*. 2022) and the possibility of a transmission vector for MLO (Pasqualetti *et al*. 2021). Recent, unpublished work will also be presented. This includes experiments on the effect of functional feeds on RMS pathology, as well as experimental induction of immunity against RMS at early life-stages to avoid pathology in large market-size fish.

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Q: have you detected RMS in another fish species?

A:Not confirmed. In farms where there are more than one species report that it is only RT which is affected. We have tried brown trout, but not real signs of infection.

Update on IHN situation in Denmark

Tine M. Iburg, Argelia Cuenca, Niccolò Vendramin

DTU-Aqua, Unit for Fish and Shellfish Diseases

Timi@aqua.dtu.dk

Denmark lost its status as IHN free Member State December 10th 2021.

Prior to lifting the IHN free status, 28 officially free compartments were established.

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

IHN was detected again April 20th at Simested Å on not previously infected farm. Epidemiological investigations were initiated, and contact farms were identified and sampled for laboratory examination by the competent authorities.

Additional eight farms were found positive (by May 24th), all most likely either infected by trade or waterborne infection. One farm that had been infected in 2021, emptied and just approved got reinfected by trade in 2022.

Importantly, in 2022, IHNV was detected also in sea cage sites, raising concern for infection of wild population and migrating wild salmon.

The status at the end of 2022 was 8 freshwater infected farms (of which 1 re-infected), 1 put and take lake and 11 saltwater farms.

All saltwater farms have been emptied by the end of the year.

In 2023, a voluntary screening program coordinated by the fish farmer association have been done besides the ordinary surveillance conducted by the competent authorities. Two small outbreaks were discovered by the screening program

Q: thanks for the update. Regarding transport of rainbow trout from Denmark, is in lorries to Germany and Netherlands where IHNV is present. How are you ensuring the IHNV is not brought back to Denmark?

A: competent authorities approves and ensures that. In principle lorries should not be moved from infected to non-infected farms, and lorries should be disinfected.

Q: why testing 20 fish? they spend quite a lot of money doing this activity, to end up having not enough number of samples per farm.

A: we agree. And we suggested to test few farms with at least 30 fish, as only 20 fish you need to have very high prevalence to catch the virus presence with only 20 fish. they are thinking that infected fish will have some clinics, however, in sea cages where the prevalence is very low it would be very difficult to detect with only 20 fish.

Comment: we should stress that this is an autonomous initiative of the industry and independent of our surveillance activities.

Comment: the tracks transporting RT should not bring more fish afterwards, so the possibility is infection in trucks is quite low. Do we know more about that?

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Summary of the IHNV outbreak in Finland in 2017-2018

Tuija Kantala and Tuija Gadd

Finnish Food Authority

tuija.kantala@foodauthority.fi

In November 2017, infectious hematopoietic necrosis virus (IHNV) was detected for the first time in Finland. Until that, Finland had maintained an official IHNV-free status. IHNV was isolated from rainbow trout (*Oncorhynchus mykiss*) in a sea-cage farm situated in the Bothnian Bay in the municipality of Ii. The sample was taken as a routine sample in a risk-based surveillance program. Epidemiological investigation led to numerous inspections and samplings, and six more IHNV positive rainbow trout establishments were detected in 2017–2018. No symptoms were reported in the positive fish.

The second IHNV case was detected in the neighbouring winter storage net cage farm of the first positive fam in the beginning of December 2017. Later in December 2017, third case was detected in a government-owned broodstock and nursery facility in continental Finland in the municipality of Tervo in Northern Savonia, in the River Kymi basin. When testing the contact farms of this facility, the fourth case was detected in a catch and release fishing pond close to the positive facility in the end of December 2017. In January 2018, the fifth and sixth cases were detected in samples from two different fishing ponds in River Vuoksi basin in the city of Nurmes and in the municipality of Kaavi. The seventh positive farm was detected in May 2018, when IHNV was detected in the brains of frozen, gutted rainbow trout from an on-growing net cage farm in the Bothnian Bay. In addition, IHNV was detected in rainbow trout escapees caught close to the positive farms in the Bothnian Bay. In total, four containment zones were established around the positive farms: in the municipality of Ii in Northern Ostrobothnia, in the municipality of Tervo in the River Kymi basin, and in the city of Nurmes and in the municipality of Kaavi in the River Vuoksi basin. All affected farms and ponds were emptied and about 230 tons of fish were slaughtered by the end of February 2018. All farms were disinfected by the end of October 2018, following a fallowing period of at least six weeks before taking new fish.

Increased number of official control visits and sampling was continued in the contact farms and other production units of the six positive farms, nearby units, units within the same water area, and units located near the units with planted fish that originated from the positive facility in Tervo. In addition, wild fish from areas with high risk of infection were sampled and tested. All samples were negative for IHNV. According to epidemiological investigation, the infection was most likely first introduced to the broodstock and nursery farm in Tervo and was then spread from there to the second positive farm in the Bothnian Bay and to the fishing ponds. To the farm where the virus was first detected in the Bothnian Bay, it likely spread from the second positive farm via water. However, the origin of the virus could not be determined.

The aim was to eradicate IHN from the four zones as quickly as possible, by surveillance program of two years. During the surveillance program, production units of farmed fish were inspected and tested twice a year, and, in addition, wild fish were also sampled and tested. All samples tested during the surveillance program were negative for IHNV. On September 15th, 2021, all four zones had regained an official IHNV-free status.

This presentation was not held as the speaker could not participate due to Covid.

Overview of IHN and VHS outbreaks in Croatia and their management since 2005

D. Oraić[1], S. Zrnčić[1], I.G. Zupičić[1], D. Brnić[1], A. Cuenca[2], N. Vendramin[2]

[1]*Croatian Veterinary Institute, Zagreb, Croatia*

[2]*DTU Aqua National Institute of Aquatic Resources, Kgs. Lyngby, Denmark*

oraic@veinst.hr

Abstract

Croatia has a long history of fish viral diseases monitoring while the Laboratory for fish pathology of the Croatian Veterinary Institute has started to participate in the surveillance of VHS and IHN according to the "Decree on the measures of animal health protection against infectious and parasite disease" issued yearly by Ministry of Agriculture, in 2000. During the period of the first five years, sporadic positive findings of infectious pancreatic necrosis virus (IPNV) were noted. However, in January 2005, an IHN occurred in imported rainbow trout fry (*Oncorhynchus mykiss*) held in the quarantine of a fish farm in the mountain region. A partial stamping out and disinfection of basins with infected fish was performed. All basins with older categories of fish were submitted to continuous controls for one year for the presence of the virus. It seems that the measures applied prevented the further spreading of the IHN virus.

Unfortunately, the same farm experienced an outbreak of VHS soon after Croatia joined the EU in July 2013. During the same year, in December, there was an outbreak of IHN at a distant and completely non-connected rainbow trout farm. Thorough epidemiological investigations of both outbreaks were undertaken but it was not possible to find out the source of infection in any of these two outbreaks. Although measures aiming to prevent the spreading of the virus were lifted, in May 2014, a mixed infection of VHS and IHN was diagnosed in the facility designated for the repopulation of open waters with brown trout (*Salmo trutta*). Following this outbreak, several other farms in different parts of Croatian territory supplied by rivers belonging to either Danube or Adriatic watershed became infected with IHNV or VHSV; in November IHNV was detected in fish from the farm on the same river as the outbreak in May, and another outbreak of mixed infection on the farm without water connection with them. The farm affected by IHN in 2013 performed stamping out while other farms were banned to transport live fish from their farms and they could sell only eviscerated market-size fish. These outbreaks significantly influenced the quantity of produced rainbow trout in Croatia and the production decreased from almost 1000 tons to 300 tons.

The surveillance program has continued and in 2018, a sample collected at the farm being positive for IHNV in November 2014 tested positive for VHSV. The last occurrence of the IHN was noted in an experimental marine site for rainbow trout farming in early spring 2020.

All samples were analysed using the same procedure: the pool of organs (anterior kidney, spleen and heart) were used for virus isolation on EPC and BF-2 cell lines and identification by ELISA followed by RT qPCR and amplification and sequencing of partial sequencing of nucleocapsid and glycoprotein genes for VHSV and „mid-G" region of the G gene of the IHNV. The obtained sequences of IHNV belong to the IHNV genotype E and VHSV to VHSV genotype Ia, subtype 2. Phylogenetic analysis of sequences of both viruses showed similarity with viruses from European countries proving that viruses have been circulated in the country.

Q: you changed from targeted to passive surveillance, do you have any ideas of what would be the output of that?

A: 20 farms are negative so far. We have tested lots of samples and there are only negative

Q: How many where fully eradicated.

A: Only one farm because it went to bankruptcy

Molecular tracing of IHNV in Europe

Argelia Cuenca, Niccolò Vendramin, Tine Iburg, Niels J. Olesen

[1]*DTU Aqua National Institute of Aquatic Resources, Kemitorvet 202, Kgs. Lyngby,*

arcun@aqua.dtu.dk

Abstract

In this presentation we will show data from some relevant infectious hematopoietic necrosis virus (IHNV) outbreaks in Europe in the last five years, and the molecular tracing of those outbreaks in the global phylogeny of IHNV.

This work presents unpublished data from different the different cases, and more coauthors than the mentioned in the title of this abstract should be included for each case. The cases revisited in the presentation include:

1) **The molecular tracing of IHNV in Finland in 2017-2018.** Isolates from the first time IHNV was detected in Finland were full genome sequenced. Phylogenetic analyses clearly show that this constitutes the first introduction of IHNV genogroup U into Europe.
   This work was done together with Tuija Kantala and Tuija Gaad from the Finish Food Authority.

2) **Molecular tracing of IHNV in North Macedonia**. IHNV has been detected in North Macedonia since 2017, and a monitoring program has been done by the Food and Veterinary Agency of North Macedonia. Here we present some results of this program, as well as the molecular tracing of recent outbreaks of IHNV in the country.
   Food and Veterinary Agency of North Macedonia. Data from this case were kindly provided by Aleksandar Trajchovski at the Faculty of Veterinary Medicine-Skopje, Ss Cyril and Methodius University.

3) **IHNV outbreak in Denmark**. In this case we will revisit the outbreak of IHNV in Denmark in 2021 and additional data gathered in 2022 and 2023. Molecular tracing among farms will be shown as well as the placement of the Danish IHNV sequences in the global phylogeny of IHNV. Heike Schütze and Uwe Fischer from Friedrich-Loeffler-Institut in Germany have kindly provided some of the data used in this part.

First detection of Piscine Orthoreovirus genotype 3 (PRV-3) in the Netherlands at a rainbow trout farm, July 2022

Olga Haenen[1], Tine Moesgaard Iburg[2], Michal Voorbergen-Laarman[1], Betty van Gelderen[1], Marc Engelsma[1], Argelia Cuenca[2], Niccolò Vendramin[2], Niels Jørgen Olesen[2]

1) *NRL for Fish Disesases, WBVR, Houtribqeg39, 8221RA Lelystad, the Netherlands*
2) *EURL for Fish Diseases, DTU AQUA, Henrik Dams Allé, Bygning 205 DK-2800 Lyngby , Denmark*

olga.haenen@wur.nl

Abstract

In the start of July 2022 the Dutch NRL for Fish Diseases at WBVR was consulted because of an acute mortality of 40% in pond rainbow trout of 15-27 cm at a fish breeding farm in the center of the Netherlands. The water temperature was 18°C, and there was less flow through because of algae that weeks. The fish originated from a farm in the very south of the Netherlands, which imported eyed eggs from Denmark with a health certificate.

At autopsy at WBVR, the fish showed lethargy, was pale, and had light exophthalmos. There were some *Glossatella* on the skin, but not on the gills. The gills were pale and congested with excess of slime, and with thickened gill tops. The liver was pale-yellowish, and the gut content also yellowish. No other abnormalities nor other parasites were recorded. Also, no muscular hemorrhages were seen. Given the signs, the Veterinary Authority was contacted with a suspicion for notifiable fish diseases.

Virus isolation of a 10% organ suspension on FHM and BF-2 cells (6-well plates) at 15°C showed cytopathic effect after 3 days, but in the second passage this did not convincingly replicate further in both cell lines. Testing the 10% organ suspension and the (sterile filtered/non-filtered) cell sub of the 1st passages with our qPCR tests for VHSV, IHNV, IPNV, EHNV, SAV and ISAV resulted in negative results. Although bacteriology revealed a *Flavobacterium* species infection of the skin and internal organs, we suspected (another) salmonid virus, given the anamnesis, autopsy, and cell culture findings. EURL was contacted for help, and kindly accepted.

EURL received 5 samples, i.e. the 10% organ suspension, and (non-/filtered) supernatant of 1st passage at FHM and BF-2 cells. They inoculated BF-2, EPC, CHSE and FHM cells with the 5 samples (24 well plates), for 2x7 days at 15°C. No cpe was seen. Moreover, EURL performed qPCR assays for VHSV, IHNV, IPNV, RANAVirus, Piscine Orthoreovirus, PRV-1, PRV-3, and a conventional PCR for OMV.

EURL found no cpe in cell culture. From all PCRs, only the Piscine Orthoreovirus and the PRV-3 qPCRs were positive: Piscine Orthoreovirus genotype 3 (PRV-3) was detected in samples 1, 4, & 5, and suspected for in 2 & 3.

We conclude, that this was the first case of PRV-3 in the Netherlands, and this was combined with flavobacteriosis. In Denmark it is known that PRV-3 can be involved in cases of increased mortality often as part of a multi-factorial disease, like this casus.

Q : do you have CPE in cells or it is something else?

A:I think it is toxic effect

Virulence of recent Czech IHNV and VHSV isolates assessed on three lines of rainbow trout

Pojezdal L[1], **Motlova J**[1], **Matejickova K**[1], **Minarova H**[1,2], **Palikova M**[2,3], **Mendel J**[3].

[1]Veterinary Research Institute, Brno, [2]University of Veterinary Sciences Brno, [3]Mendel University in Brno

pojezdal@vri.cz

Abstract

The presence of viral haemorrhagic septicaemia or infectious hematopoietic necrosis is sporadically detected in farmed rainbow trout in the Czech Republic, usually due to presence of patho-anatomical signs of the disease and increased mortality. In 2019, an IHNV strain with presumed low virulence was isolated from the ovarian fluid of a clinically healthy rainbow trout spawner.

Virulence of the isolate was tested on rainbow trout fingerling (circa 3cm) during a 30-day infection trail. Two isolates of VHSV and an additional IHNV isolate from 2010 were administered in a bath challenge with a titre of 5,2x10^3 to 2,36x10^5. Survival of the fish varied from 0% (VSHV-2021) to 27% (IHNV-2010) and the results did not confirm the proposed low virulence of the IHNV2019 isolate, possibly due to the age difference between the trial fish and animal of isolate origin.

Another trial was focused on the potential resistance of specific rainbow trout lines against IHNV and VHSV. Three defined trout lines were used – Denmark N30, Denmark N29 and Italy N15. The line DK-N30 was bred for a QTL for resistance against *Flavobacterium* infection. Immersion challenge of one IHNV and one VHSV isolate (titre $1x10^{\wedge 2}$ and $1x10^{\wedge 4}$, respectively) was performed. Mortalities, specific antibodies against the viruses, leucocyte counts, haematocrit, haemoglobin, phagocytic activity, and interferon activity were measured and compared, with only a few parameters showing statistical differences between the three rainbow trout lines.

This study was supported by the Ministry of Agriculture of the Czech Republic grant MZE-RO0523 and the project PROFISH CZ.02.1.01/0.0/0.0/16_019/0000869 financed by ERDF in the operational program VVV MŠMT.

Q: How do you select your QTL line? Is that secret? Who discovered?

A: I have not been able to know what is in the QTL – that may be secret Was developed by a company at Czech republic

Q: you have a very strong reduced survival in the first trial whereas there is very mild in the second trial, what is that?

A: Size of the fish, and virus titer changed

Comment: There is big variation from trial to trial, that needs to be taken in consideration when assessing viral virulence.

Q: in the vhsv trial you saw a decrease in the hemoglobin and in erythrocites. Did you see clinical findings that may explain this decrease?

A: Yes, all the typical clinical signs were present in each group.

Q: have you thought in using transcriptomics?

A: For sure, that would be the next steps, together with gene expression and transcriptomics

Comment: we are looking for a qtl for resistance of ihnv and vhsv and we have already done that for RTFS (chromosome 25). We have also done it for WSD and furunculosis and vibriosis.

SESSION III Results from ongoing research on listed and emerging fish diseases

Chair : Morten Schiøtt

Unexpected cross reactivity of MAb IP5B11 used for VHSV diagnostics resolved.

Niels Lorenzen[1], Niels Jorgen Olesen[1], Tohru Mekata[2] and Takafumi Ito[2]

[1]*National Institute for Aquatic Resources, Technical University of Denmark, Kemitorvet 202, 2800 Kgs Lyngby, Denmark*
[2]*Pathology Division, Fisheries Research Agency, Fisheries Technology, Institute, 422-1 Nakatsuhamaura, Minami-Ise, Mie 516-0193, Japan*

Corresponding author: nilo@aqua.dtu.dk

Abstract
The monoclonal antibody (MAb) IP5B11, which is used worldwide for the diagnosis of viral haemorrhagic septicaemia virus (VHSV) in fish, reacts with all genotypes of VHS virus (VHSV) without any cross reactions with other fish viruses - with one exception: back in 1995, strong binding of MAb IP5B11 to a rhabdovirus isolated from an endemic trout population in Lake Garda in Italy was reported (1). No or limited cross reaction with other VHSV-specific antibodies suggested that the virus belonged to an undescribed viral species and it was named *carpione rhabdovirus* (CarRV) according to the host species know as "carpione" or *Salmo (trutta) carpio*. Apart from Western blotting demonstrating binding of MAb IP5B11 to the CarRV N protein, the exceptional cross reaction remained to be resolved until recently. Following next generation genome sequencing of CarRV and N protein sequence alignment including five species of fish novirhabdoviruses, the epitope recognized by mAb IP5B11 has now been identified (2). Dot blot analysis confirmed the epitope of mAb IP5B11 to be associated with the region N219 to N233 of the N protein of VHSV. Very few amino acid positions appeared to make the epitope unique to VHSV – and CarRV. Phylogenetic analysis identified CarRV as a new member of the fish novirhabdoviruses and the appropriate name should therefore be *Novirhabdovirus carpione.*

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2. Ito, T., Mekata, T., Olesen, N. J., & Lorenzen, N. (2023). Epitope mapping of the monoclonal antibody IP5B11 used for detection of viral haemorrhagic septicaemia virus facilitated by genome sequencing of carpione novirhabdovirus. Veterinary Research, 54(1), 35.

Comment : Farming difficult, high pressure on eggs, 2-3 farms. Do not test lake so do not know if virus is in the lake

Q: What's the story of the virus?

A: Lake Garda once connected to sea 10.000 years ago. Perhaps this virus is ancient. What is the history of these novirhabdoviruses? Developed in fish?

Q: Other salmonids in the lake?

A: Yes, but no mixing

Q: Coevolution with virus?

A: Yes, perhaps

Q: How to do molecular clock on virus

A: Possible

Pilot assessment of economic impact of IHN outbreaks on Danish Fish Farms

Britt Bang Jensen[1], Ása Maria Olsen[1], Niccolo Vendramin[1], Torsten Snogdal Boutrup[2]

1: DTU-AQUA, 2: AquaPri

abrj@aqua.dtu.dk

Abstract

During the years 2021 and 2022, a total of 21 outbreaks of Infectious Haematopoietic Necrosis (IHN) were reported in Danish aquaculture. As IHN has not previously been present in Denmark, there is a lack of knowledge as to how an outbreak of IHN affects the Danish Aquaculture farm.

Thus, we have conducted a small-scale study with the aim of assessing the mortality associated with IHN outbreaks in Denmark.

We retrieved production data from two Danish rainbow trout producers for their 2022 production. For freshwater production, data were retrieved on a farm level, and thus we were able to compare mortality and weight gain between IHN-infected and non-infected fish on a farm level. For the seawater production, data were received on cage level, and we compared IHN-infected cages to non-infected cages.

Data from 2 IHN-infected and 2 non-infected freshwater farms were included. The cumulative mortality of the two IHN-infected freshwater farms reached 29.1 and 35.9%, respectively, whereas the cumulative mortality for the two non-infected farms was significantly lower, at 0.8 and 13.8%, respectively.

Data from sea cages included 62 IHN-infected cages and 22 non-infected cages. The average accumulated mortality for the IHN-infected cages was slightly higher than for the non-infected cages, at 0.43 and 0.35%, respectively. This difference was non-significant.

The economic impact of IHN in the sea farms was low or non-existent, as both the mortality and the biomass losses were insignificant. For the two freshwater farms that experienced IHN, losses could be divided into the cost of kg feed lost in mortality and lost sales (fish that died before reaching market size). For the two farms, the total losses equalled 32.807 DKK (~4.400€) and 43.651 DKK (~5.860€), respectively. In these numbers were not included increased work hours or potential downgrading of the fish.

In December 2021, Denmark revoked its status as IHN-free. According to the Animal Health Law, the control and mitigation of infectious diseases are, to a large extent, directed by the needs and ambitions of the farmers. Thus, economic assessments of the impacts of diseases provides a tool for them to assess the costs and benefits of control.

We plan to conduct a more thorough study of the biological and economic impact of IHN on Danish Aquaculture.

Comment: In Denmark one of the biggest challenges is infection with Aeromonas. It appeared that IHN affects also the consequences of Aeromonas infection both in terms of mortality and effectiveness of antibiotic treatment, this should be taken in account.

Q: Initial weight influences the output of an infection.

A: Should take variation into account.

Temperature influence on IHNv replication and pathogenicity in rainbow trout

Toffan[1] A, Marsella[1] A., Buratin[1] A., Pascoli[1] F., Abbadi M., Toson[1] M., Cuenca[2] A., Vendramin[2] N.

[1]*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy*

[2]*Technical University of Denmark, Lyngby, Denmark*

atoffan@izsvenezie.it

Abstract

In recent years, Infectious Hematopoietic Necrosis has spread in Europe, with outbreaks occurring in previously disease-free countries such as Denmark and Finland. The strain of IHNv responsible for outbreaks in Denmark has been isolated from infected fish in follow-up sampling at water temperatures close to 20°C. The IHNv genotypes U and M can replicate in cell culture and induce disease in rainbow trout, *Oncorhynchus mykiss*, and sockeye salmon, *O. nerka*, at temperature higher than 14°C. This feature has never been demonstrated for European IHNv strains, which belong to genotype E. A selection of IHNv strains, including the Danish and some Italian isolates, were analyzed for their replication fitness at different temperatures of incubation. The panel of isolates was subjected to titration by end-point dilutions assays on EPC cell monolayers at 15°C, 20°C, 22°C and 25°C. Cytopathic effects were monitored daily and titers were calculated according to the Spearman-Karber formula daily up to seven days post inoculation. Based on the results, a subset of IHNv strains has been selected for an *in vivo* challenge in order to evaluate the influence of water temperature on their pathogenicity in rainbow trout juveniles kept at 12°C and 19°C. The titers yielded in the *in vitro* testing showed a high degree of variability of IHNv strains in relation to their sensitivity to temperature. The Danish isolate and some Italian strains replicated without significant reduction when incubated at 22°C and could replicate even at 25°C. Notably, the temperature tolerance appears linked to the environment of origin rather than to the year of isolation. I*n vitro* analysis demonstrate a wider temperature range for IHNv strains belonging to genotype E and will be validated by the *in vivo* testing. Based on results obtained a change of paradigm in the surveillance of IHN should be considered also at the regulatory level.

Funded by the Italian Ministry of Health IZSVe RC 13/19 and by the Aquaexcel 3.0 TNA programme.

Comment: Nice talk. Recent outbreak in Netherlands was at 18C

Q: Uwe: No immune system in cell culture. Do you look into the immune response in your in vivo experiment?

A: We will look at the viral load, but not on the immune response.

Q: Temperature is essential. Viral genetics at protein level. What parts are more adapted to temperature?

A: to be investigated.

Elucidating the dynamics and transmission potential of the aquatic pathogen *Renibacterium salmoninarum* in Rainbow trout

Alejandra Villamil Alonso[1], Giulia Zarantonello[1], Niccolò Vendramin[1], Lone Madsen[1], Tine Moesgaard Iburg[1], and Argelia Cuenca[1]

[1]*National Institute for Aquatic Resources DTU Aqua, Technical University of Denmark, Section for Fish and Shellfish Diseases*

alvalo@aqua.dtu.dk

Renibacterium salmoninarum (Rs) is a facultative intracellular bacterium and the aetiologic agent of bacterial kidney disease (BKD). This chronic infection is associated with severe mortality in salmonid fish worldwide. Rainbow trout (*Oncorhynchus mykiss*), the main species farmed in Denmark, is highly susceptible to Rs although the mechanisms of transmission and the chronic state of the infection are not yet fully understood. Notably the disease represents one of the main health challenges in Recirculating Aquaculture systems (RAS).

Our study aimed to characterize disease kinetics and host survival through *in-vivo* cohabitation challenge at 6°C and 12°C in RAS. Rainbow trout shedders were intraperitoneally injected with Rs (100µl, $1x10^9$ CFU $fish^{-1}$, $1x10^8$ CFU $fish^{-1}$) or saline water (control), and subjected to survival analysis during 12 weeks. Naïve fish were put in cohabitation with shedders to assess the transmission potential and bacterial kinetics through qPCR of bacterial DNA and environmental DNA (eDNA) extracted from fish kidney and water samples, respectively. Moreover, bacteriological examination was performed by plating fish kidney, spleen, and brain on SKDM agar to confirm the presence of Rs.

All challenged groups were susceptible to Rs, but only the shedders experienced reduced survival due to BKD. Reduced survival began at an earlier stage at 12°C, and the probability of surviving until the end of the trial was 16.10%, 5%, and 0% for the groups injected at low dose at 12°C, high dose at 12°C, and high dose at 6°C, respectively. Infection by cohabitation was established within 2 weeks, and abundance of bacterial transcripts was significantly higher at 4 weeks post infection in the kidney of sampled fish in all infected groups. Interestingly, water eDNA analyses revealed bacterial shedding at its highest at 3 weeks post infection and detected the bacteria at late infection sampling points. Moreover, Rs was reisolated in SKDM media from the kidney of some fish also at the latest stage of infection at 6°C.

Our results provide information on disease progression and transmission potential of BKD and insights on the chronic state of the infection. Here we also confirm the possibility to detect Rs in RAS at different infection stages. Overall, this study defines an infection model of Rs in rainbow trout, essential to further explore the underlying mechanisms of pathogenicity of the bacterium.

Comment: If skin barrier is not disturbed during cohabitation you will not get high mortality. Compromising skin integrity will probably get you different results.

A: Thanks

Q: What is high and what is low dose? How did you determine it?

A: *R. salmoninarum* grow slowly on agar. We did a small pilot study previously, high and low correspond to a tenfold dilution

Q: How did you detect R.salmoninarum in water samples.

A: Relatively high load of bacteria in water already after one week.

Q: Difference between 6 and 12 C in eDNA?

A: Some. Higher amount of bacteria late in the trial in the 6 C group, but probably related to a delayed mortality in the 6 C group.

Comment: No replication in water, then better preserved at low temperature.

Early immune response of two common carp breeds to koi herpesvirus infection

Machat R[1], Pojezdal L[1], Gebauer J[1], Matiasovic J[1], Tesarik R[1], Minarova H[1,2], Hodkovicova N[1], Faldyna M[1]

[1] *Veterinary Research Institute, Brno,* [2] *University of Veterinary Sciences Brno*

pojezdal@vri.cz

Abstract

Common carp is the most economically important fish farmed in the Czech Republic. The presence of koi herpesvirus in the country was confirmed in the early 2000s and 0 to 16 outbreak sites are detected and eradicated every year since. Although the factors of resistance of various carp breeds against the virus were studied extensively, including infection trials and mapping of quantitative trait loci, evolving analytic methods present an opportunity to further characterize the reaction of the immune system of carp to the infection.

Koi carp, previously confirmed as highly susceptible to KHV, and amur wild carp (confirmed resistant) were selected for this study and intraperitoneally infected with low virulence KHV strain. Viral load, expression of selected immune genes (qPCR) and changes in expression profile of proteins (mass spectrometry) were monitored in head kidney, spleen and gills of the fish 3 and 7 days post infection.

Although the viral loads increased during infection, no mortalities were observed. Few significant difference were detected at the gene expression levels, but protein expression revealed earlier onset of parameters related to class I interferon signalling pathway and complement cascade in amur carp compared to common carp. This might suggest the immunological foundation of increased amur carp resistance to KHV.

This study was supported by the Ministry of Agriculture of the Czech Republic grant MZE-RO0523 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 IV:Update from the EURL

EURL TRAINING COURSE FOR 2023

Niccoló Vendramin and Tine Moesgaard Iburg

EURL Fish Diseases, National Institute of Aquatic Resources, Technical University of Denmark, Denmark

niven@aqua.dtu.dk and timi@aqua.dtu.dk

Abstract

In 2022, two training course were hold at the premises of DTU AQUA in Kgs, Lyngby.

A short overview of the program and the activities will be presented.

The EURL team will also present the plans for the period of the WorkProgram 2023-2024

In Week 41 of 2023, we will hold the course on "Surveillance for listed fish disease".

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

RESULTS OF THE PROFICIENCY TEST, PT1 AND PT2, 2022

Teena Vendel Klinge, Niccoló Vendramin, Argelia Cuenca

EU Reference Laboratory for Fish and Crustacean Diseases,

DTU AQUA National Institute of Aquatic Resources, Kemitorvet, Bygning 202, 2800 Kgs. Lyngby, Copenhagen,

niven@dtu.vet.dk

Abstract

A comparative test of diagnostic procedures was provided by the European Union Reference Laboratory (EURL) for Fish Diseases. The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2).

PT1 was designed to primarily assess the identification of the fish viruses 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. This report covers both the results of PT1 and PT2. Participants were asked to identify the content of each ampoule by the methods used in their laboratory which should be according to the procedures described in EURL diagnostic manuals available on the website

Participants were asked to download an excel sheet from the EURL web site (http://www.eurl-fish.eu/) to be used for reporting results and to be submitted to the EURL electronically. Additionally, participants were requested to answer a questionnaire regarding the accreditation status of their laboratory.

The tests were sent from the EURL in September 2022.

The test was divided into proficiency test 1 (PT1) and proficiency test 2 (PT2).

42 laboratories participated in PT1 while 41 participated in PT2.

Each laboratory was given a code number to ensure discretion. The code number of each participant is supplied to the respective laboratories with this report. Furthermore, the providers of the

proficiency test have included comments to the participants if relevant. An uncoded version of the report is sent to the European Commission.

Résumé and concluding remarks PT1

90% of the parcels were delivered by the shipping companies within two week and 100% was delivered within 26 days.

Overall 32 out of 42 participants scored 100% success rate; out of the 10 laboratories which underperformed only one participant scored <100% for the sole reason that they did not back up their concluding results of ampoule II (ECV) with sequencing. 5 laboratories did not find the virus in ampoule V (low titre IHNV). 2 laboratories had a contamination in ampoule II (SVCV) and one laboratory answered EHNV in ampoule II even though the sequence show ECV. Suggestions to improve on underperformance will be provided individually to each laboratory.

Résumé and concluding remarks PT2

41 laboratories participated in PT2, 37 obtained 100% success rate. Out of the 4 laboratories which underperformed, three obtained a lower score because did not provide sequencing for the ISAV isolate in ampoule VIII. This point will be addressed directly with the participants that has underperformed.

All 41 laboratories correctly identified the CyHV-3 (KHV) in ampoule VII.

All 41 laboratories correctly identified the ISA virus in ampoule VIII, hereof three laboratories did not sequenced. One laboratory wrongly concluded that the isolate was HPR0 ISAV.

36 laboratories tested for SAV and all correctly identified the virus in Ampoule IX, five laboratories did not test for SAV.

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

EURL for Fish Diseases, work done in 2023

Niccolò Vendramin and Britt Bang Jensen

DTU Aqua

niven@aqua.dtu.dk

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.

The 26th Annual Workshop of the National Reference Laboratories for Fish Diseases was held in hybrid form, using for the virtual part the zoom platform, on 30th and 31st of May 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.

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 annual proficiency test for fish diseases (PT) was divided into PT1 and PT2 with 42 laboratories participating. The tests were sent from the EURL 5th of October 2022. 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 2022 and the EURL has focused on improving the diagnostic manual for ISA, IHN and VHS, and finalise the manual for EHN.

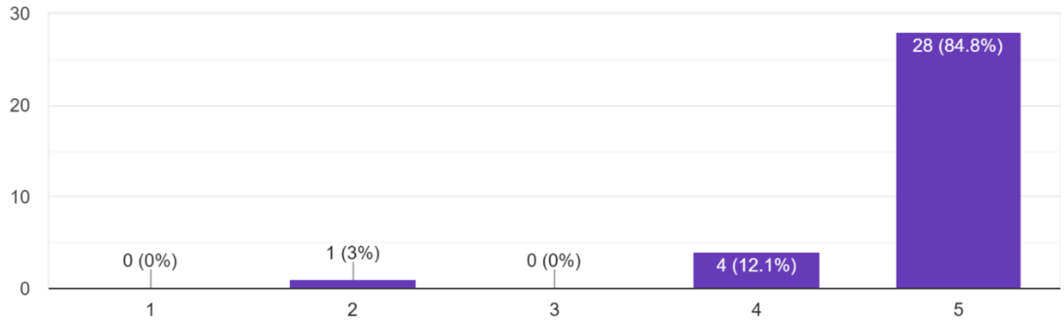
During 2022, resources were again used to collate data on surveillance, health categorisation and diagnostics in EU; to identify and characterise selected virus isolates; to type, store and update a library of listed virus isolates; to supply reference materials to NRLs; to provide training courses in 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 33 questionnaires retrieved is shown below.

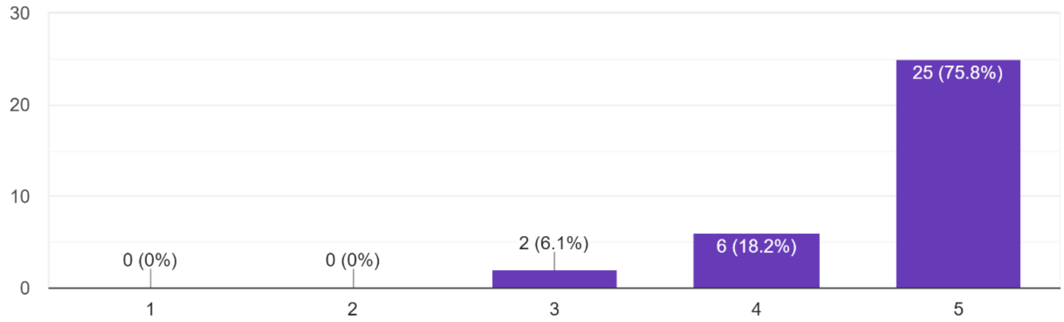
Session I: Update on important fish diseases and their control - Quality of presentations

33 responses



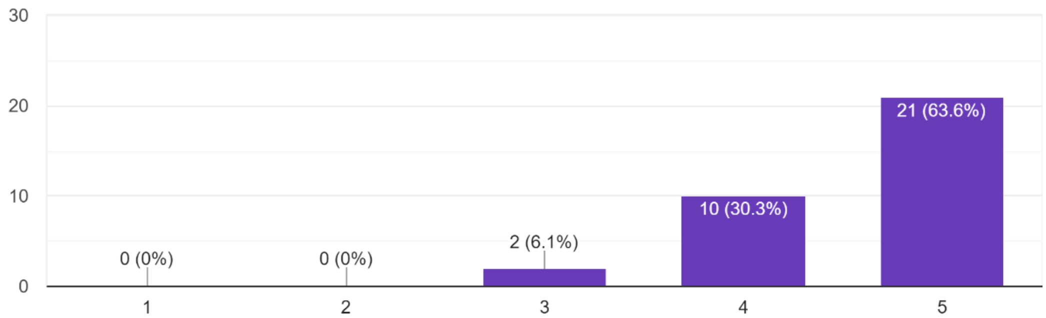
Session I: Update on important fish diseases and their control - relevance for you

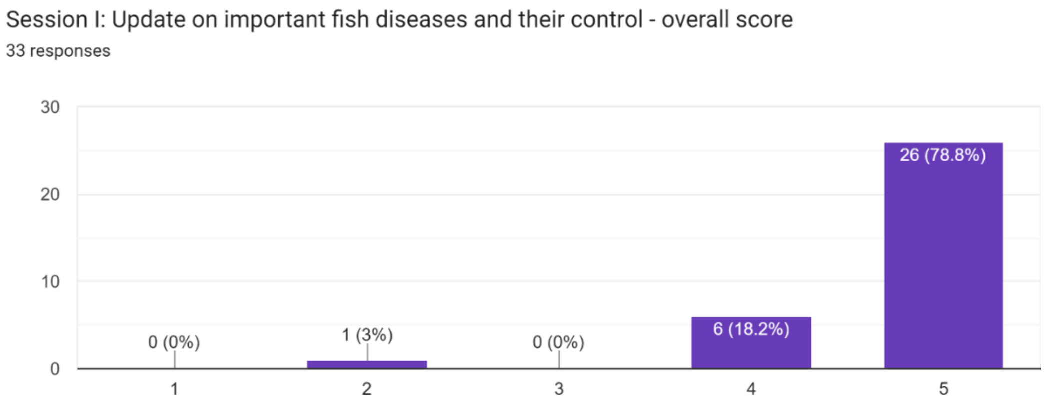
33 responses



Session I: Update on important fish diseases and their control - increase of your knowledge

33 responses





Session I:

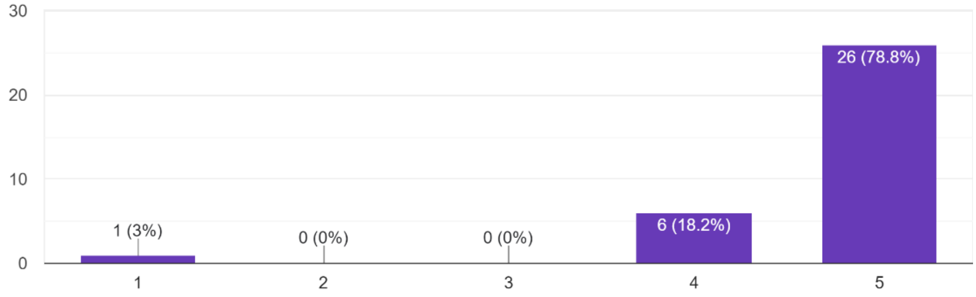
Update on important fish diseases and their control - comments, remarks, inputs6

responses

- very valuable presentations
- Nice, PRV-3 was discussed already, and in total in 2 lectures.
- Very well organized
- no comments
- Could the speaker(s) consider reducing the amount of text in the presentations, especially if they are just reading out what is written in the slide
- A very well devised program and very beneficial

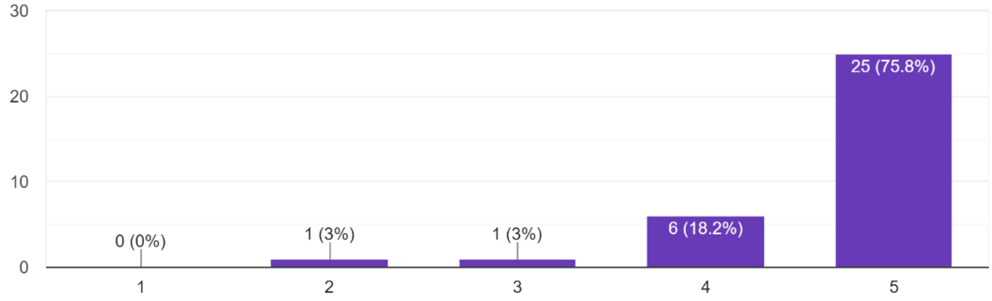
SESSION II: Control and Surveillance of fish diseases in Europe- Quality of the presentations

33 responses

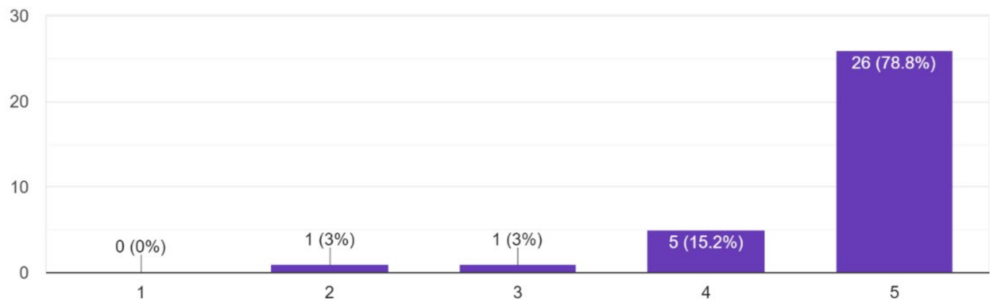


SESSION II: Control and Surveillance of fish diseases in Europe- relevance for you

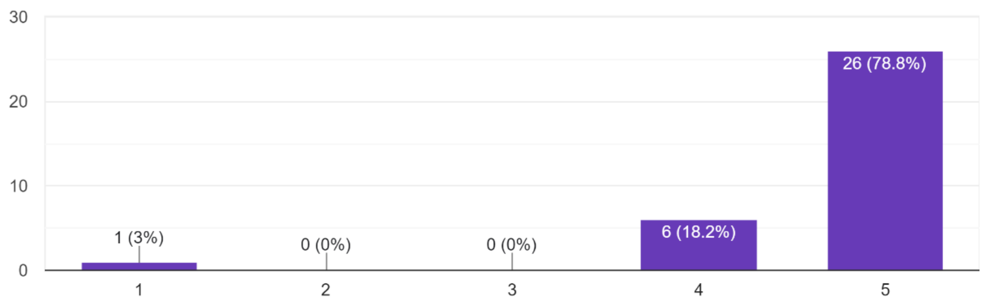
33 responses



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



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

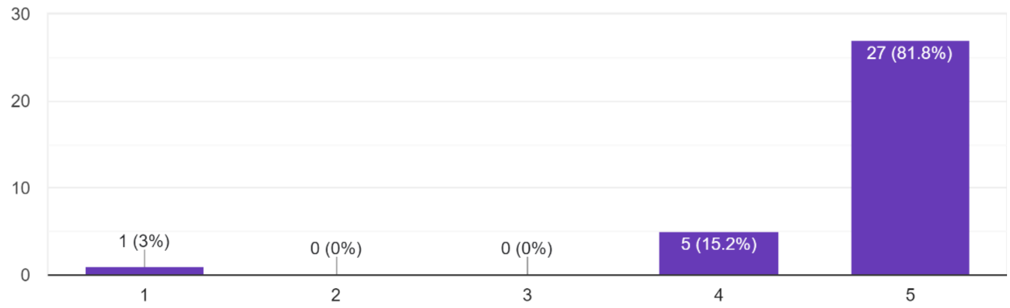


SESSION II: Control and Surveillance of fish diseases in Europe- comments, remarks inputs5 responses

- Well done!
- Relatively much on IHN, understandable and important. And learnt on Perch Rhabodvirus disease and RMS.
- Very interesting, informations on Practical issues
- No
- Some very interesting presentation which gave a lot of food for thought for future work and collaborations

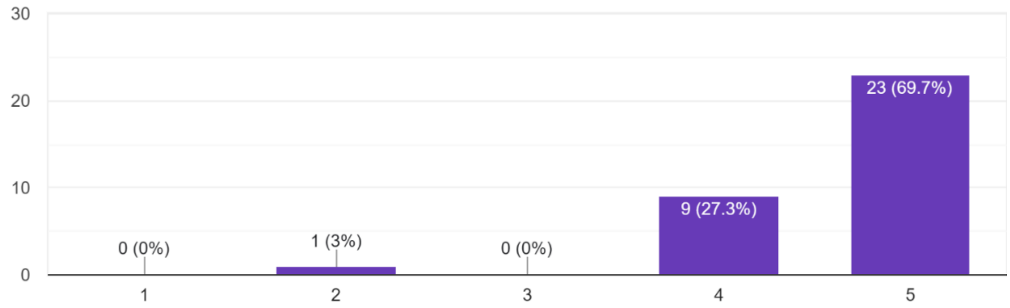
SESSION III: Results from ongoing research on listed and emerging fish diseases-quality of the presentations

33 responses



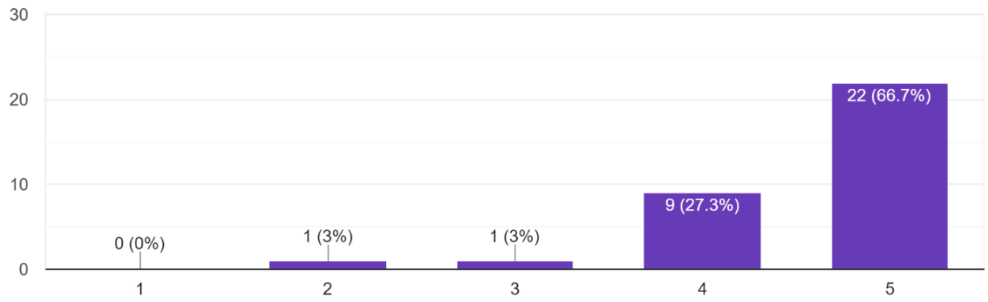
SESSION III: Results from ongoing research on listed and emerging fish diseases-increase of your knowledge

33 responses



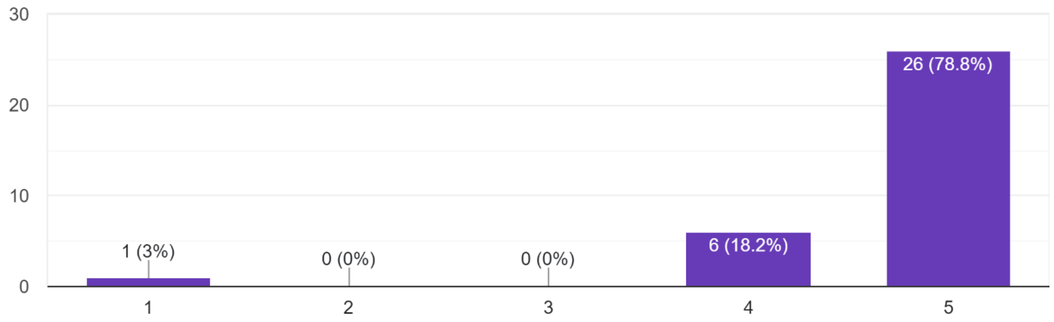
SESSION III: Results from ongoing research on listed and emerging fish diseases-relevance for you

33 responses



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

33 responses

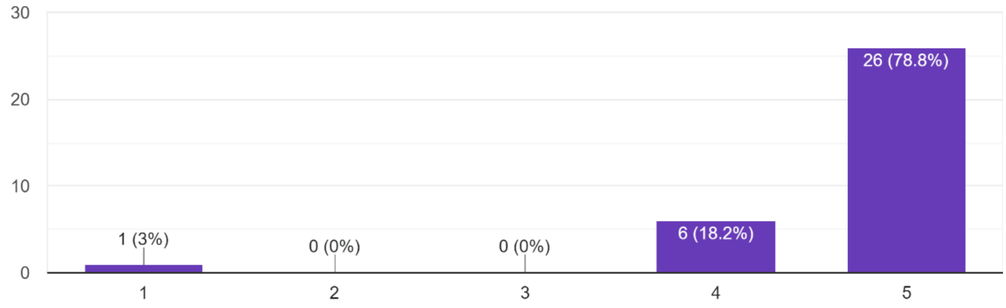


SESSION III: Results from ongoing research on listed and emerging fish diseases- comments, inputs, remarks7 responses

- Well done!
- very interesting talks, regarding various pathogens; reports on in vivo experiments and in vitro assays always of interest.
- Learnt on the carpione rhabdovirus. Nice. IHN also relevant!
- Kind request to add at least one presentation referring to diseases of Mediterranean fish species
- no comments
- No
- Very good presentations

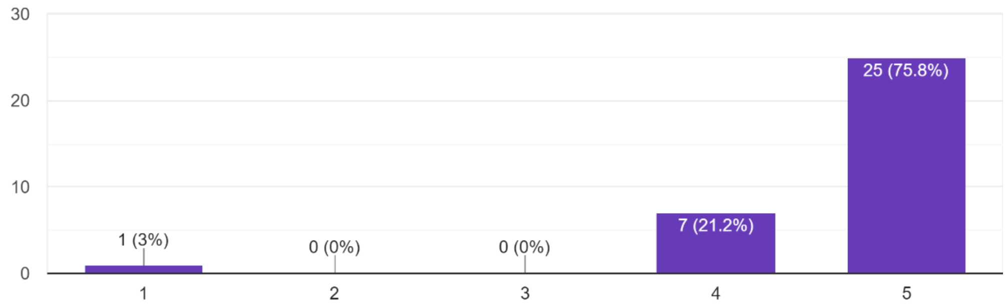
SESSION IV: Update from the EURL for fish diseases- quality of the presentations

33 responses

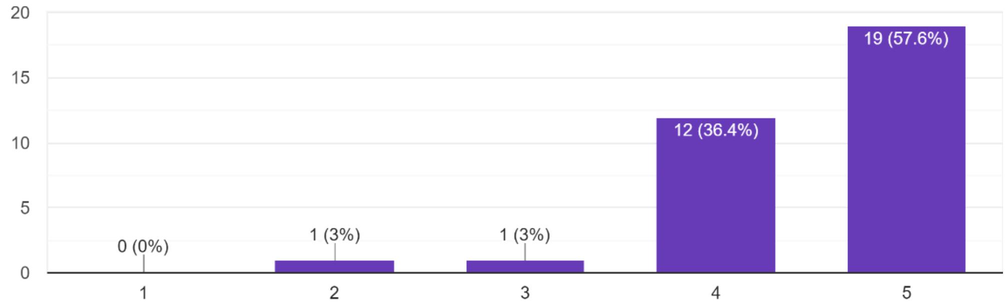


SESSION IV: Update from the EURL for fish diseases- relevance for you

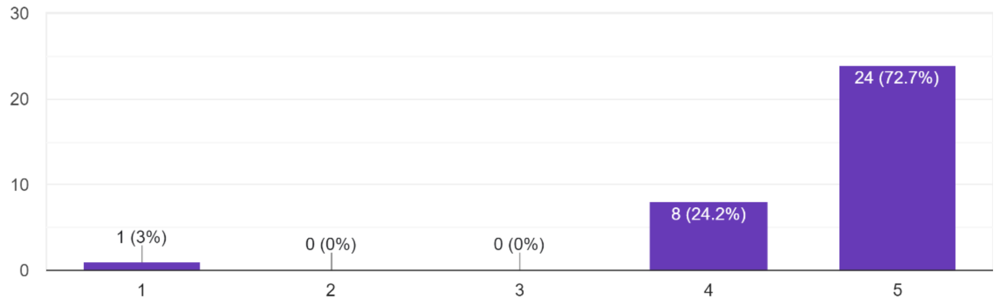
33 responses



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



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



SESSION IV: Update from the EURL for fish diseases- comments, inputs, remarks9

responses

- Well done!
- for all sessions: WIFI would be nice... for all sessions: there is much weight on salmon production in Norway (and then, why always Norway and not e.g. on RT in Turkey ?), which is not even a MS; maybe considering turning the focus more to the ideed very diverse aquaculture production in EU MS might be of interest for many participants
- thank you very much for this very interesting meeting...
- All fine! Thank you so much for this great NRL meeting (again)!
- Very informative
- no comments
- Reference sequence database for use in fish disease genotyping analysis please.
- Always good to have an overview of what is happening at the EURL and the training opportunities available to NRLs
- We had no internet, and I could not donwnload the abstracts pdf during the day. It would be nice to have them printed again next year.

Greetings and conclusions of the meeting

The next meeting will be held in May 2024 and we aim at having an online 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