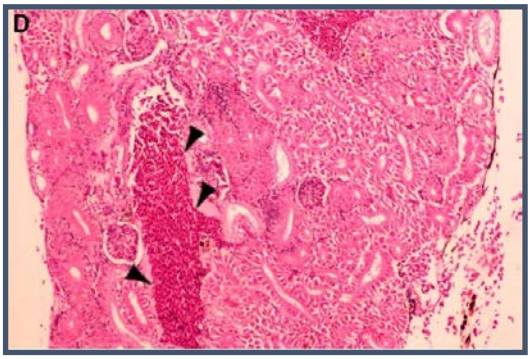
Report

21st Annual Workshop of the National Reference Laboratories for Fish Diseases

Kgs. Lyngby, Denmark

May 30th – 31st 2017



Tilapia disease in commercial hybrid tilapia (Eyngor *et al*., 2014)

Red Mark Syndrome (RMS) in rainbow trout

Organised by the European Union Reference Laboratory for Fish Diseases,

National Veterinary Institute, Technical University of Denmark, Kgs. Lyngby

Table of Contents

Introduction and short summary

The 21st Annual Workshop of the National Reference Laboratories for Fish Diseases was held 30th – 31st of May, at DTU Veterinary Institute, 2800 Kgs. Lyngby, Denmark. This annual workshop was the first to be held at our new premises in Kgs. Lyngby.

A total of 63 participants from 35 countries attended over the two days period. All presenters arrived to the workshop, thus, no last minute changes were made in the programme. There were five sessions with a total of 28 presentations, three of which were given by invited speakers; 1) Nadav Davidovich from Israel (Tilapia viruses), 2) Britt Bang Jensen from Norway (Cardio Myopathy Syndrome (CMS) in Atlantic salmon) and 3) Nicholas Stinton from United Kingdom (Use of tablet devices during aquaculture visits), a Working Group activity and a Round Table discussion. The scientific programme of the Annual Workshop was 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 and EURL coordinator, Nikolaj Gedsted Andersen. 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 2016 was provided on the basis of the results obtained from the Survey & Diagnosis questionnaire. A report compiling all information are available at the EURL website http://www.eurl-fish.eu/activities/survey_and_diagnosis. Secondly, the fish disease situation in Norway was presented; a detailed report in Norwegian is available at https://www.vetinst.no/rapporter-og-publikasjoner/rapporter/2017/fiskehelserapporten-2016. An English version will be available later. The two final presentations in Session 1 were an update on the disease situation in aquatic organisms in the Mediterranean and a presentation of aquaculture and disease threats in Australia with an update on Epizootic Haematopoietic Necrosis Virus (EHNV).

The second half of the morning was allocated to the Working Group activity, introduced for the first time during the Annual Workshop in 2014. Briefly, each participant was, before the workshop, asked to choose one of four groups, divided into fish species, and rate the different fish diseases for that certain fish species in his or hers country. All participants received beforehand four tables listing the most renowned pathogens for the most important farmed fish species in Europe. Before the workshop, each participant had the opportunity to interact with different stakeholders and assess impact on production, economy, legislative consequences and risk of future significance for the different infectious diseases in 2016. During the Working Group activity, participants discussed and agreed on a common rating for all the diseases. Each Working Group lastly presented their results to the rest of the participants at the workshop. A more detailed outcome of the Working Groups can be found later in this report under the chapter "Working Groups: Perception on the impact and risk of infectious fish diseases in Europe".

The second session of the Workshop was dedicated to emerging diseases. Firstly, a combined presentation with data from the Netherlands and Korea was given on Cyprinid Herpesvirus 2 (CyHV-2). This was followed by an update on Cardio Myopathy Syndrome (CMS) in Atlantic salmon from Norway. The following two presentations were both given by employees at DTU VET and addressed Piscine Orthoreovirus (PRV) in Europe and Red Mark Syndrome (RMS) in rainbow trout, respectively.

The third session on control and surveillance of relevant pathogens in the EU started with a presentation describing Carp Edema Virus Disease (CEVD) in Europe. This was followed by an update on the fish health situation in France. Then Niels Jørgen Olesen gave a thorough review on aquaculture in the new Animal Health Law, an update on listing of fish diseases in the EU legislation and an update on susceptible species from an OIE working group. The last part of session three was dedicated to data and handling by a presentation on how to collect and interpret disease data and secondly, how to use tablet devices in inspection and compliance visits of aquaculture facilities. In the evening of the first day, a banquet was held at Restaurant "Brdr. Price" in Tivoli.

The second and last day was opened with a session on results from ongoing research on listed and emerging fish diseases. Traditionally, this fourth session faced several different topics covering molecular characterization of pathogens, development of new diagnostic techniques, including serology, conventional PCR and Real Time PCR, cell cultures and characterization and description of new fish pathogens. The session started with three presentations addressing Infectious Salmon Anemia (ISA) from Norway and the Faroe Islands. The virus, VHS, was also addressed in this session with four presentations, mainly on molecular work and identification of virulence markers. An interesting case on recurrent unexplained mortalities in warm water fish species in Israel was also presented in this session. In another presentation, attention was given to barcoding of fish cell lines, and it was shown that the origins of these are not always correctly noted. The fourth session ended before lunch with the second activity, Round Table discussions. All participants had the opportunity to give a short pitch talk on a topic they believed needed discussion and would be of common interest. Two pitch talks were given; one on sample preservation for PCR and a second on the use of FTA cards in fish diagnostics. Both topics initiated interesting plenary discussions.

The Annual Workshop ended with the traditional fifth session on updates from the EURL. The results of the two proficiency tests sent out in 2016, PT1 and PT2, were presented. The programme and application system for the annual training courses, which will be provided by the EURL in October 2017, was described and participants were given the opportunity to suggest topics for future courses. The planned EURL activities in year 2017 were presented and proposals for the EURL work plan for 2018-2020 were discussed.

Employees from DTU VET took minutes from the meeting: Jacob Günther Schmidt, Lone Madsen, Anna Luiza Farias Alencar, Argelia Cuenca Navarro and Niccolò Vendramin. Nikolaj Gedsted Andersen has assembled a draft of the report, which has been sent to all the presenters and participants, who asked and answered questions during the presentations, for correction in order to avoid misunderstandings. Nikolaj Gedsted Andersen has finalized the report thereafter.

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 Teena Vendel Klinge, Nikolaj Gedsted Andersen and Niels Jørgen Olesen, with the help from the rest of the fish disease section at the National Veterinary Institute, DTU. The meeting next year is tentatively planned to be held at the end of May 2018, also at DTU VET. 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 Nikolaj Gedsted Andersen

Programme

Tuesday May 30th

Annual Workshop of the National Reference Laboratories

08:45 – 9:15	**Registration**
09:15 – 09:30	Welcome and announcements *Nikolaj Gedsted Andersen and Niels Jørgen Olesen*

SESSION I: Update on important fish diseases and their control

Chair: Olga Haenen and minutes: Jacob Günther Schmidt

09:30 – 09:50	Overview of the disease situation in Europe *Niels Jørgen Olesen*
09:50 – 10:10	Update on the disease situation in Norway *Brit Hjeltnes*
10:10 – 10:25	Update on the disease situation in aquatic organisms in the Mediterranean *Niccolò Vendramin*
10:25 – 10:45	Aquaculture and disease threats in Australia with an update on Epizootic Haematopoietic Necrosis Virus (EHNV) *Ellen Ariel*
10:45 – 11:05	***Coffee break***
11:05 – 12:45	Perception on the impact and risk of infectious fish diseases in Europe: Group 1: Atlantic salmon, Group 2: Rainbow trout, Group 3: Seabass and Seabream and Group 4: Cyprinids, Sturgeon, Eel and Tilapia *Nikolaj Gedsted Andersen*

12:45 – 13:35 ***Lunch***

SESSION II: Emerging diseases

Chair: Charlotte Axén and minutes: Lone Madsen

13:35 – 13:55 Cyprinid Herpesvirus 2 (CyHV-2)

Olga Haenen/Duan Hongan

13:55 – 14:15 Update on Cardio Myopathy Syndrome (CMS) in Atlantic salmon

Britt Bang Jensen

14:15 – 14:35 Piscine Orthoreovirus (PRV) in Europe

Niccolò Vendramin

14:35 – 14:55 Red Mark Syndrome (RMS) in rainbow trout

Jacob Günther Schmidt

14:55 – 15:25 ***Coffee break***

SESSION III: Control and surveillance of relevant pathogens in the EU

Chair: Uwe Fischer and minutes: Anna Luiza Farias Alencar

15:25 – 15:45 Carp Edema Virus Disease (CEVD) in Europe – an update

Olga Haenen

15:45 – 16:05 Update on the fish health situation in France

Lénaïg Louboutin

16:05 – 16:35 A: Aquaculture in the new Animal Health Law, B: Listing of fish diseases in EU legislation and C: Susceptible species – report from an OIE working group

Niels Jørgen Olesen/Knut Roenningen

16:35 – 16:55 Disease data collection in aquaculture; how to collect and interpret data

Britt Bang Jensen

16:55 – 17:15 Tablet devices in inspection and compliance visits of aquaculture facilities

Nicholas Stinton

17:30 – ***Bus transport to Hotel Cabinn Scandinavia and Tivoli***

19:30 – ***BANQUET at restaurant "Brdr. Price" in Tivoli***

Wednesday May 31st

Annual Workshop of the National Reference Laboratories

SESSION IV: Results from ongoing research on listed and emerging fish diseases

Chair: Thomas Wahli and minutes: Nikolaj Gedsted Andersen

09:00 – 09:20	Infectious Salmon Anemia (ISA) - recent development and future control *Torfinn Moldal*
09:20 – 09:40	A new case of ISA in the Faroe Islands: A diagnostic challenge? *Debes Christiansen*
09:40 – 10:00	First field evidence of the evolution of a virulent infectious salmon anaemia virus (ISAV) from a non-virulent ISAV-HPR0 progenitor *Debes Christiansen*
10:00 – 10:20	In the search for virulence markers of Viral Haemorrhagic Septicaemia Virus (VHSV) *Anna Luiza Farias Alencar*
10:20 – 10:40	***Coffee break***
10:40 – 11:00	Recurrent unexplained mortalities in warm water fish species; emergence of new tilapia viruses and epidemiological approach for surveillance *Nadav Davidovich*
11:00 – 11:20	Barcoding of fish cell lines - the origin of cell lines is not always what we believe *Niels Jørgen Olesen*
11:20 – 11:40	Validation of Viral Haemorrhagic Septicaemia (VHS) virus conventional RT-PCR *Hyoung Jun Kim*
11:40 – 12:00	New Viral Haemorrhagic Septicaemia (VHS) virus subtype in Europe *Argelia Cuenca Navarro*

12:00 – 12:30	Round Table on stability studies of samples for diagnostics
	All participants are invited to give short talks (3-5 min.) on stability studies conducted in their lab
	Preliminary suggestions:
	Pitch: Sample preservation for PCR (Argelia Cuenca Navarro)
	Pitch: Use of FTA cards in fish diagnostics (Argelia Cuenca Navarro)
12:30 – 13:30	***Lunch***

SESSION V: Update from the EURL

Chair: Niels Jørgen Olesen and minutes: Argelia Cuenca Navarro

13:30 – 13:50	Results of the Proficiency Test, PT1 and PT2, 2016
	Niccolò Vendramin
13:50 – 14:10	EURL Training Courses. Topics and organization for courses 2017
	Nikolaj Gedsted Andersen and Tine Moesgaard Iburg
14:10 – 14:30	EURL activities in 2016
	Niels Jørgen Olesen
14:30 – 14:50	EURL work plan for 2017 and 2018 – ideas and plans?
	Niels Jørgen Olesen
14:50 – 15:00	Next meeting and end of 21st Annual Workshop
	Niels Jørgen Olesen
15:00 –15:30	***Coffee, cake and goodbyes***

SESSION I: Update on important fish diseases and their control

Chair: Olga Haenen

Overview of the disease situation and surveillance in Europe in 2016

N. J. Olesen[1] and Niccolò Vendramin [1]

[1]*DTU Vet National Veterinary Institute, Kemitorvet 202, Kgs. Lyngby, njol@dtu.vet.dk*

Abstract

The Questionnaire on Surveillance and Diagnosis (S&D) which is collated annually is the only comprehensive overview of the disease situation in aquaculture in Europe. The information has been made available on the EURL web site (www.eurl-fish.eu), where all raw data can be obtained. Last year the S&D was changed significantly, where we asked each Member State to write a report and submit it to us together with few questions. This year the same template was submitted but asking only for changes since the previous year avoid duplicating earlier information. The questionnaire comprises 3 parts:

1. General data on aquaculture fish production: Number of fish farms, and the health categorization according to Council Directive 2006/88/EC, and information on national surveillance programmes.
2. Epidemiological data on the disease situation in each Member State with focus on the listed diseases (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.

Production data from FEAP

The data on the European aquaculture production was this year obtained from the "European aquaculture production report 2007-2015" Prepared by the FEAP secretariat October 2016. Again this year we validated the data against the FIGIS database and concluded that there were no major discrepancies except for the common carp production estimated by FEAP to be only 1/3 of the production data we obtained from FIGIS. The report does not include information on the number of fish farms, and therefore these data were obtained directly in the questionnaire. The report only provides data from back to 2015 as data from 2016 will only be available in autumn 2017. The total fish production in aquaculture in Europe increased again both in 2014 and in 2015 after a decrease in 2013 and is now at 2,359,705 t, the highest level ever. The increase however is almost only due to increases in non-EU Member states. Among the Member states the production has been almost horizontal in the past 10 years with a 30.000 t increase in 2015 to 674,493 t. The Atlantic salmon production, account for 1,57 mill ton against 1.55 mill ton in 2014, and is by far the largest contingency in Europe. The rainbow trout production is again below 400 000 t after steady increases in the previous years. The decrease is due to reduced production of table size rainbow trout, while production of large rainbow trout is increasing. After several years of increased production Turkey have experienced an almost 20% reduction from 2013 to 2015 but are still the largest contributor of table size rainbow trout with > 100 000 t production. The carp production is still mainly in the Eastern part of Continental Europe and is very stable with 57.610 t produced in all. Both the production of sea bream and especially sea bass also increased in the Mediterranean countries with a production of 147.640 t and 158.479 t, respectively. Among other fish species of interest are eel (with 6.266t in 2015 no significant increase since 2010), sturgeon (2.559 t) and caciar with 20% increase from 2014 to 127 t, turbot (decrease from 12.748t to 7.823t in 2013 and increase again to 11.270 in 2015), the cod production have collapsed from 22.729t in 2009 to 78t in 2015. The production of cleaner fish as lumpfish for lice control is increasing significantly but the total production has not been possible to retrieve.

Health categorization of fish farms:

Many countries provided very clear and correct answers and almost all Member States did reply to the questionnaire when compared to the previous year's providing a rather complete overview of the status of fish health categorization in Europe. This year in all 12.680 farms with susceptible species were included in the questionaire as categorized while a total of 30.810 fish farms were reported. The number of categorized farms is unfortunately very variable from year to year which more reflects changes in the way of reporting than de-facto changes. There was a significant increase in the reported number of farms in categorized zones and compartments (From 8.505 in 2012 to 14.508 in 2015 for VHS and from 7.360 in 2012 to 12.130 in 2015 for

KHV!) this was especially due to Germany who successfully included almost all their farms in categorized zones.
74% of the authorised trout farms in Europe are situated in category III zones for VHS and 70% for IHN, with 23% and 27% respectively in Category 1. For both diseases the remaining 3% of the farms are situated in category II, IV or V. In all countries except Norway almost all salmonid farms are in Category I for ISA with 73% in Category I and 27% in category III. Only very few carp farms are approved KHV free in Category I (1%) and almost all are placed in Category III (96%) or in Category II 3%.
There are still several different views on how categorisation shall be performed, e.g. should VHS free marine rainbow trout farms be placed in Category III or I? Considering the risk of infection with VHSV from the marine environment.
Commission Decision 2015-1554 provide the guidelines for obtaining disease-free health statuses with regard to ISA and to contain infection with HPR deleted ISAV, saying that detection of Isavirus HPR0 will not compromise the health status of a fish farm and is not notifiable to the EU (in contrast to OIE where detection of ISAV HPR0 is still notifiable). Some Member states do not include small registered APBs in the categorisation (e.g. hobby farms) but according to 2006/88/EC Annex III health categorisation comprise all APBs in the Member states, zones and compartments for each category. Only fish species listed as susceptible for the given listed disease shall be included in the categorization. Therefore important aquaculture species as sea bass, sea bream, meagre, eel and pike-perch are not included in the European health surveillance for specific diseases.
The new Animal Health Law has now been adopted and includes all aquatic animals; in this connection the categorisation system will be simplified and be made more transparent.

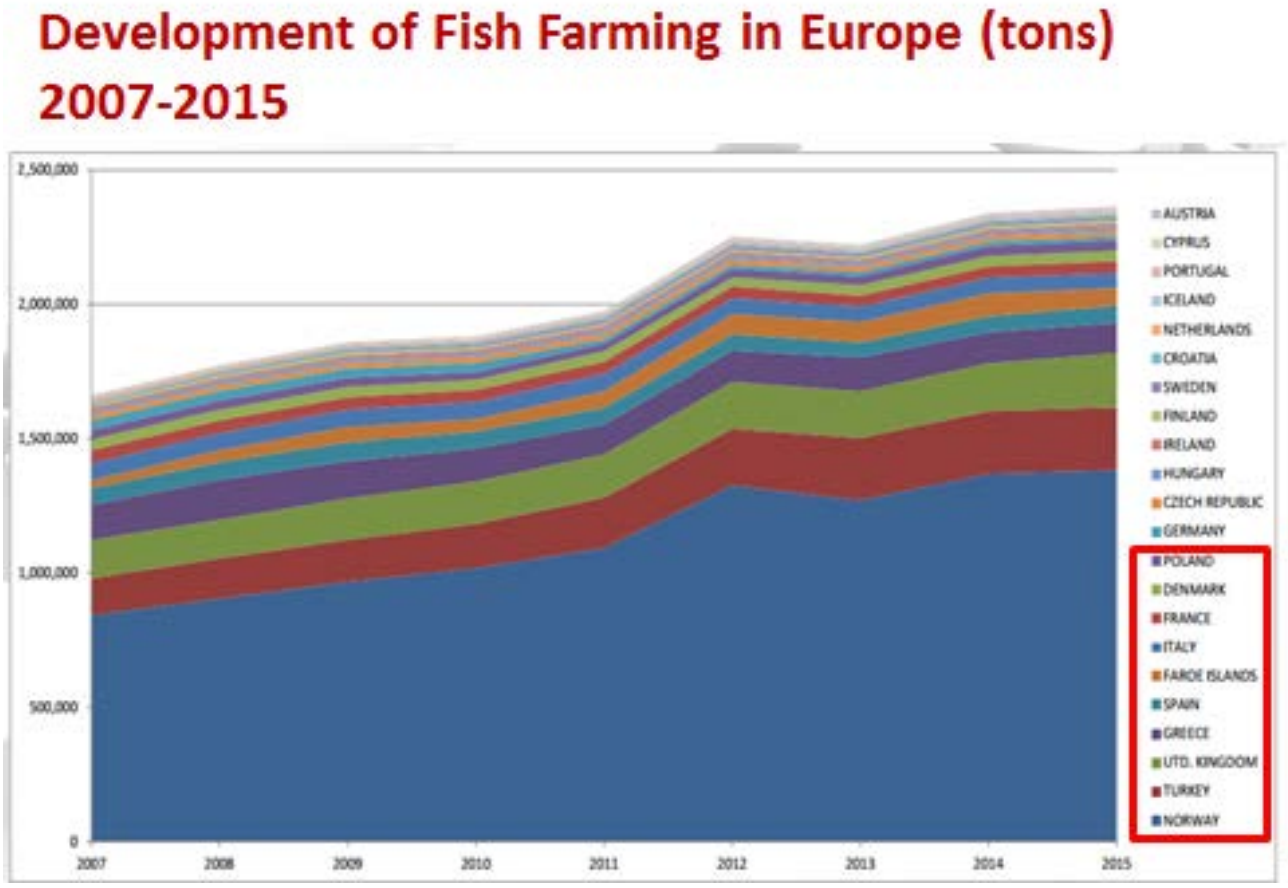
Outbreaks and severity of listed diseases in Europe

Concerning the epidemiological data on the non-exotic diseases a moderate increase in the number of **VHS** infected farms and outbreaks were observed in Belgium (in brook trout farm!), Bavaria (9 new cases), a new outbreak of VHS in Romania and 3 outbreaks in Czech Republic. Decrease in severity observed in Mecklenburg-Western Pomeranian and in saxony, and only 1 farm positive in Switzerland no other reports of changed severity of VHS was given no VHS positive samples found in Croatia.
For **IHN** only few reports were given: increase in Saxony with 3 IHNV outbreaks without losses, 1 new IHN outbreak in the Netherlands, no new outbreaks in Croatia.
For **ISA** Norway reported 12 new sites with ISAV HPRΔ and Faroe Islands reported 1 finding of HPRΔ in 2016 without clinical symptoms and with an outbreak in 2017 no other report on ISA.
Concerning **KHV** Germany reported increases in number of infected farms in Saxony and Rhineland-Palatina. In ireland and in Lithuania 1 outbreak in garden ponds reported, in the Netherlands 2 outbreaks in open water carps. In England and Whales 33 outbreaks were reported in 2016 (only 11 in 2015). In Croatia KHV was reported from 2 farms and 2 ponds for sport fishing- Croatia have not encountered KHV before.

Other fish diseases problems in EU

A whole range of other disease problems in 2016 were reported:
In **rainbow trout** the major concerns are flavobacteriosis (RTFS), red mark syndrome, puffy skin, enteric redmouth, and infectious pancreatic necrosis but also, lactococcosis, bacterial kidney disease, proliferative kidney disease, ichthyophtariasis, saprolegniosis, columnaris and furunculosis (especially in brown trout).
In **salmon** farming it is sea lice, pancreatic disease, heart and skeletal muscle inflammation, cardiomyopathy syndrome, amoebic gill disease, and moritella and in addition flavobacteriosis, furunculosis, and saprolegniosis (Baltic salmon). In norway detection of PRV-*om* have been made in more than 100 samples.
In **pike-perch** farming 1 outbreak of perch rhabdovirus with 100% mortality in larvae.
In **cleaner fish** it is primarily vibriosis and *A. salmonicida* infection giving problems
In **Carp** it is primarily *Aeromonas hydrophila,* SVC (in Romania)
In **seabass** and **seabream** it is primarily VNN/VER, *Lernathropus kroyeri* infection, Microcotylosis and Rash syndrome
In northern European countries the most common problems in the salmon production are thus sea lice, PD, and AGD, in addition several countries reported finding of Winter Ulcer Disease in salmon caused by *Moritella viscosa.* In continental Europe it is primarily bacterial diseases like ERM and Aeromonas infections, AGD

and RTFS – but also red mark syndrome is causing severe problems. Parasite infestations as Ich is still a very serious problem especially in view of the foreseen prohibition of use of formalin, while problems in the Mediterranean countries are the same as in continental except for Lactoccocosis wich is more common in Southern Europe and Nodavirus infection in mariculture which definitely plays an important role and as a bottleneck for especially the seabass production.
There are very large differences between countries on how many samples are tested on cell cultures, ranging from < 100 to several thousands. Annex 5 provide the total number of laboratory examinations conducted in Europe in 2016 on VHSV, IHNV, ISAV, KHV, SVCV, CEV, IPNV, SAV, and Nodavirus, respectively.



Questions and comments:

Uwe Fischer: *"Is there a listing on arctic char for VHS and IHN?"*

Niels Jørgen Olesen: *"I am part of a working group in OIE on char. Char should be under legislation. Decision will be made next year, but most likely we will recommend including arctic char on the list."*

Update on fish disease situation in Norway 2016

Brit Hjeltnes, Geir Bornø, Mona Dverdal Jansen and Cecilie S. Walde .

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

Abstract

In 2016, Norway produced 1.171200 tons of Atlantic salmon (*Salmo salar*), 84500 tons of rainbow trout (*Oncorhynchus mykiss*) 6-7000 tons of Atlantic cod (*Gadus morhua*), 1600 tons of Atlantic halibut (*Hippoglossus hippoglossus*), 500 tons Arctic char (*Salvelinus alpinus*) and 2-300 tons turbot (*Scophthalmus maxima*).

Salmon lice (*Lepeophtheirus salmonis*) infestation represents one of the most significant challenges to Norwegian aquaculture and increased resistance to anti sea lice chemicals is a problem of great concern. The fish farming industry, the Norwegian government and research institutions are investing in the development of non-chemical methods to control sea lice. Cleaner fish eating sea lice are used by a large number of fish farmers. In 2016, fish health personnel reported that mechanical de-licing resulted in an increased level of mechanical injury and death in treated fish.

Infection with salmonid alphavirus (SAV) remains the most serious virus disease in sea-farmed salmonids. In total, 138 new sea-farms were registered affected in 2016, a level similar to 2015.

Infectious salmon anaemia (ISA) was diagnosed in 12 farms in 2016 compared to 15 farms in 2015. The majority of the affected farms were situated in Nordland and several of these farms appear to have been infected with a closely related strain of virus.

Infectious pancreatic necrosis (IPN) was diagnosed in 27 salmonid farms in 2016. This is a slight reduction from 2015, but clearly lower than the peak year of 2009 when IPN was diagnosed in 223 farms. Use of QTL strains of salmon combined with increased focus on eradication of ‘house strains’ of virus is probably the most important reasons behind the reduction in number of cases in recent years.

Heart and skeletal muscle inflammation (HSMI) was in 2014 removed from the Norwegian national list of notifiable diseases. Reported cases from the Norwegian Veterinary Institute, NVI (101) and private laboratories (101) indicate a similar situation in 2016 as in 2015.

Cardiomyopathy syndrome (CMS), also known as ’heart rupture,’ was diagnosed by NVI on 90 sites. Considering reported cases from private laboratories (108 cases), this indicates an increase over recent years.

While AGD (*Paramoeba perurans*) remains an important parasitic infection, the disease was not as severe in 2016 as it was in 2014. Gill disease occurs during all phases of salmonid culture. Chronic gill inflammation is a particularly significant and recurring problem. Bacterial ulcers continue to be a problem in farmed fish particularly in northern Norway. Yersiniosis (*Yersinia ruckeri*) continues to affect an increasing number of farms and in recent years, there appears to be an increasing trend towards clinical outbreaks in large sea-farmed salmon.

Production losses remain a significant problem in Norwegian aquaculture.

Questions and comments:

Vlasta Jencic: *"Why do you not do Yersiniosis vaccination?"*

Brit Hjeltnes: *"Because we find it in hatcheries in small fish and in recirculating systems, so early problems in young fish."*

Uwe Fischer: *"Do you know what the marker for IPN resistance in rainbow trout is?"*

Brit Hjeltnes: *"In Atlantic salmon they found a super strong marker site. A QTR."*

Niccoló Vendramin: *"PRVom in rainbow trout? Have you seen the disease?"*

Brit Hjeltnes: *"No. We have surveillance, but see no clinical disease. Only detection of virus."*

Update on the disease situation in aquatic organisms in the Mediterranean

Niccolò Vendramin

[1]*National Veterinary Institute, Technical University of Denmark, Denmark*

niven@vet.dtu.dk

Abstract

The Mediterranean basin represents an interesting area for aquaculture. The production in the area is quite composite, over than historically established salmonid (rainbow trout, brook trout and charr) and carp farming, Mariculture (sea cages aquaculture) has developed in the last 20 years.

The aim of this initiative, which started in 2012, is to set up a platform that can link authorities and stakeholders aiming to target the main sanitary issues in the basin and focus future research activities on these topics.

A simple questionnaire asking to rank the three most important diseases for marine and fresh water sector was delivered to a panel of experts.

Contributions from 17 experts were obtained about disease situation in the Mediterranean basin for 2016. Data will be presented and discussed showing comparison with previous years focusing both on important known diseases and emerging pathogens.

Data and presentation will be uploaded on the website of the EURL for fish diseases at the following link: http://www.eurl-fish.eu/Activities/annual-meetings

N. Vendramin, S. Zrncic, F. Padrós, D. Oraic, A. Le Breton, C. Zarza & N. J. Olesen. EAFP bullettin volume 36(1),2016)

Questions and comments:

None

Aquaculture and disease threats in Australia with an update on Epizootic Haematopoietic Necrosis Virus (EHNV)

Ellen Ariel

College of Public Health, Medical and Veterinary Sciences

James Cook University, Townsville 4811, QLD Australia

Ellen.Ariel@jcu.edu.au

Abstract

The Australian continent spans from tropical regions to cool temperate and the aquatic species cultured reflect the local water temperatures. Southern bluefin tuna (*Thunnus maccoyii*) and Atlantic Salmon (*Salmo salar*) are cultured in the southern seas, while prawns (*Penaeus monodon, Marsupenaeus japonicus*), edible oysters (Pacific- *Crassostrea gigas* & Sydney rock – *Saccostrea glomerata*) and barramundi (*Lates calcarifer*) dominate the culture in the northern coastal regions. Pearl oysters (*Pinctada maxima*) are also cultured in these tropical areas. The freshwater crayfish industry is a nascent industry. Rainbow trout (*Oncorhynchus mykiss*) and pike perch (*Perca fluviatilis*) originally imported from Europe, thrive in similar environments in Australia. Both are susceptible to EHNV which occurs sporadically in freshwater fish in the south-eastern parts.

Questions and comments:

Tomas Vesely: *"EHNV is from what part of the world? Some jump from other species?"*

Ellen Ariel: *"I don't know. Some native Australian fish are susceptible. Don't know about jump. Could be jumping from a native Australian species, but I am speculating here."*

Uwe Fischer: *"Escapees of Atlantic salmon and rainbow trout do they survive in nature?"*

Ellen Ariel: *"Yes and yes."*

Olga Haenen: *"What about EHN mortality in perch? Maybe hard to estimate in wild perch."*

Ellen Ariel: *"We see very high mortality in outbreaks and also in experimental infection. And morbidity in rainbow trout."*

Niels Jørgen Olesen: *"We are using a lot of resources on EHNV in Europe also. It is part of the proficiency test. We will see what the listing of EHNV will be in the future."*

Britt Bang Jensen: *"In the US they have massive die offs from Rana virus. We have to have surveillance or we will put wild species at risk."*

Working Groups: Perception on the impact and risk of infectious fish diseases in Europe

Group 1: Atlantic salmon, Group 2: Rainbow trout, Group 3: Seabass and Seabream and Group 4: Cyprinids, Sturgeon, Eel and Tilapia

Nikolaj Gedsted Andersen

National Veterinary Institute, Technical University of Denmark, Copenhagen, Denmark

ngaan@vet.dtu.dk

Abstract

The overall aim of this part of the 21st annual workshop is to evaluate the impact and risk of relevant fish diseases in Europe. This year, not only impact in production, economy and legislative consequences will be evaluated, but also risk of increasing significance in the future. Oppose to last year, where groups where divided into European regions this year, we have divided the groups into fish species.

The participants will be divided into 4 groups; Group 1: **Atlantic salmon**, Group 2: **Rainbow trout**, Group 3: **Seabass and Seabream** and Group 4: **Cyprinids, Sturgeon, Eel and Tilapia**. A facilitator has been assigned to each group (Group 1; Brit Hjeltnes, Group 2; Richard Paley, Group 3; Niccoló Vendramin and Group 4; Niels Jørgen Olesen).

The participants will receive a table beforehand (found in the workshop folder) which lists the most relevant diseases for the respective fish species, and this table must be used in the rating process. Furthermore, each group has to assign a presenter for presenting the agreed results for the rest of the participants (cannot be the facilitator). Thus, the tasks are to discuss the important fish diseases, provide an agreed score and select the 5 most important fish diseases, present these most important diseases and describe why they have been selected as the most important.

Time schedule:

- 11:05 – 11:10 Nikolaj Gedsted Andersen will explain how to use the tables
- 11:10 – 11:15 Dividing into groups by “show of hands”
- 11:15 – 11:25 Each participant uses 10 minutes to fill in the table
- 11:25 – 11:30 Groups will join and move to location
- 11:30 – 12:15 Evaluation and rating of fish diseases (each facilitator will be given the table in A3 size to fill out an overall table representing the groups agreed decision)
- 12:25 – 12:45 Plenary presentation from the groups (5 minutes each)

Working group's summary

In order to integrate data provided through the questionnaire on Survey and Diagnosis in Europe with direct inputs from the NRL (National Reference Laboratories) representatives, we arranged this Working Group activity. It is the fourth time, starting in 2014, that we conduct this activity during the Annual Workshop. As opposed to last year, when groups were formed according to geographical region, this year, the groups were formed on the basis of fish species. The participants were divided, after own choice, into one of these four groups:

Group no.	Fish species	Facilitator
1	**Atlantic salmon and cleaner fish**	Brit Hjeltnes
2	**Rainbow trout**	Richard Paley
3	**Seabass and Seabream**	Niccoló Vendramin
4	**Cyprinids, Sturgeon, Eel and Tilapia**	Niels Jørgen Olesen

At first, participants were asked to fill out a table with a list of relevant diseases for each fish species with a view to the sanitary status in their home countries. For each disease, the participants were asked to give a score on four different parameters characterizing the impact of the disease:

1) The perception of the impact on production, meaning the severity of losses in terms of mortalities, reduction of growth, etc.

2) The impact on the economy, indicating the cost of prevention (i.e. vaccination and biosecurity measures), treatment and reduced value of the product.

3) Consequences due to trade restrictions, national plans for control/eradication, suspension time after antibiotic treatment etc.

4) The risk of increasing significance in the future.

In order to allow each Member State representative to prepare for the Working Group activity, the material was sent out by email 10 days prior to the Annual Workshop.

Based on discussions, each group was asked to agree on a score (from 1-10) for each of the four parameters for each disease, select the five most important diseases for each fish species and to select a representative to describe the outcome of the work for the other participants of the Annual Workshop.

The output of the Working Groups was:

Group 1; Atlantic salmon and cleaner fish

The group agreed that the most important fish disease for Atlantic salmon was the crustacean parasitic **sea lice**. This major threat was appointed a high score (>6) in all four categories; 1) impact on production, 2) impact on economy, 3) legislative consequences and 4) risk of increasing significance in the future. As noticed in last year's identical session, the sea lice are still considered the bottleneck for future development of the salmon production in Norway. The economic impact of this disease is mainly related to increased resistance to treatment.

The disease scoring second highest (5-10) was **pancreas disease (PD)** caused by a viral infection of the salmon alphavirus (SAV). Due to national legislation in some areas of Norway, there is a special focus on this disease and it scored 10 in the legislative impact category (0 for countries where the disease is not relevant).

The viral disease, **heart and skeletal muscle inflammation (HSMI) disease,** scored third highest (2-5) with the highest score (5) under risk of increasing significance in the future and **cardiomyopathy syndrome (CMS)** disease were appointed the fourth highest score. HSMI and CMS were agreed to be quite similar in the rating in all four categories. The viral disease, **infectious salmon anemia (ISA)**, the bacterial disease, **atypical vibriosis,** and the parasitic disease, **amoebic gill disease (AGD),** shared the fifth place with almost the same rating in all categories, expect for ISA, which had a high score (9-10) under legislative consequence. The situation with ISA is relatively under control, but may increase in recirculation systems with smolt production in the future. AGD is important in Ireland and Scotland.

The situation with cleaner fish is difficult to assess due to lack of data. The main issue is flavivirus in Norway and Scotland. There is a range of bacterial issues when cleaner fish are kept together with Atlantic salmon, but problems are low when they are kept alone. Cleaner fish are e.g. susceptible to AGD, but it is only a problem when they are kept together with the Atlantic salmon.

Group 2; Rainbow trout

A prioritized list was difficult to make, since some important viral diseases are only found in certain countries and absent in others. However, the most important disease was agreed to be the **viral hemorrhagic septicemia (VHS) virus** with a high score (5-10) in all categories. The second most important disease was decided to be **infectious hematopoietic necrosis (IHN)**. The score for VHS and IHN were almost identical and both diseases must be considered important in rainbow trout production in Europe.

After the viral diseases VHS and IHN the most important diseases are bacterial, mainly **rainbow trout fry syndrome (RTFS)** caused by *Flavobacterium psychrophilum*. This disease is followed by **enteric redmouth disease (ERM)** caused by *Yersinia ruckeri* and **furunculosis** caused by *Aeromonas salmonicida*. As opposed to the viral diseases, the bacterial diseases are found in almost all countries producing rainbow trout. Parasites are generally less important.

It was also noted that it is important to discriminate between wild and farmed fish. In wild fish, parasitic diseases such as proliferative kidney disease (PKD) and fungi, such as saprolegniasis, may be more important.

Group 3; Seabass and Seabream

For European seabass, **viral encephalopathy and retinopathy (VER),** also known as **viral nervous necrosis (VNN),** was considered the most important disease. As last years working group concluded, this disease is still a bottleneck for further development in the aquaculture of seabass, due to the high impact on production and economy. Following VER-VNN were the diseases **tenacibaculosis,** caused by *Tenacibaculum maritimum*, the parasitic crustacean, *Lernanthropus latis*, **aeromoniasis** and **pasteurellosis,** caused by *Photobacterium damselae* subsp. *Piscicida* (especially important in Spain). Pasteurellosis is not important if the fish are properly vaccinated.

For gilthead seabream the most important diseases were considered to be the parasite *Sparicotyle chrysophrii*, followed by **red rash** which is of unknown aetiology. Third was the parasitic disease, **enteromyxosis,** caused by the myxosporean parasite *Enteromyxum leei*. Ranged fourth and fifth by the group, were **pasteurellosis** and VER-VNN, respectively.

Group 4; Cyprinids, Sturgeon, Eel and Tilapia

For cyprinids the ranging of important diseases was as follows; 1) **koi herpesvirus disease (KHVD), cyprinid herpes virus (CyHV-2)**, **carp edema virus (CEV)**, **spring viraemia of carp (SVC)** (only found in some countries) and **Aeromoniasis**. KHVD and CEV both got the highest score (5) for the cyprinids, in the category "risk of increasing significance in the future".

For sturgeon the general score was low (<6) for all diseases. **White sturgeon iridovirus (WSIV)** was agreed to be the most important disease overall, and likewise the disease with the highest score (5) in the category "risk of increasing significance in the future". Following were problems with *Flavobacterium* sp., **freshwater white spot disease** caused by the parasite *Ichthyophthirius multifiliis* and herpesvirus.

For eel, the general score was, like for sturgeon, also low. Viral **anguilla herpesvirus 1 (AngHV1)** was the most important disease with medium scores (5) for impact on production and economy. Following were *Pseudomonas anguillarum*, **Vibriosis**, the viral disease **infectious pancreatic necrosis (IPN)** and *Edwardsiella tarda*.

For Tilapia, only four diseases where ranged. Most important were **tilapia lake virus (TiLV)** followed by **Tilapia parvo-like virus (TPLV)** and problems with *Streptococcosis* sp. and *Francisella* sp. infections. TiLV and TPLV are considered to have medium to high risk of increasing significance in the future, both with a score of 7 in this category.

SESSION II: Emerging diseases

Chair: Charlotte Axén

Cyprinid herpesvirus 2 (CyHV-2)

Olga Haenen

National Reference Laboratory for Fish Diseases, Wageningen Bioveterinary Research of Wageningen UR, P.O. Box 65, 8200 AB Lelystad, The Netherlands

Abstract

Cyprinid herpesvirus 2 (CyHV-2) is known as the causative agent of herpesviral haematopoietic necrosis of goldfish *Carassius auratus auratus*. Recently, the virus has also been detected from Prussian carp *C. gibelio* and crucian carp *C. carassius* from various European and Asian countries.

This lecture deals with three subjects regarding CyHV-2:

1. Importation of CyHV-2 infected goldfish from third countries into the Netherlands and characterization of strains: Ito et al., submitted (based on joint work of **T. Ito and J. Kurita** from Tamaki Laboratory NRIA, Mie, Japan and **O. Haenen**)
2. Cyprinid herpesvirus 2 (CyHV-2) infection in wild gibel carp, *Carassius auratus gibelio* in the Netherlands (**O. Haenen, M. Engelsma, T. Ito**)
3. A primary cell line from allogynogenetic silver "crucian" carp *(Carassius auratus gibelio)* brain (CrCB) (**Duan Hongan** from Lianyungang Entry-exit Inspection and Quarantine Bureau, Jiangsu Province, P.R.China)

1. Four of eight flight imported goldfish batches from Asia and the Middle East into the Netherlands were CyHV-2 positive by PCR, of which one was from a CyHV-2 disease case at a Dutch importers site. Results on sequence analysis of the mA region of CyHV-2 strains from these and previous cases showed that there were at least six different lengths ⇨tentatively at least four genotypes. Results on virus isolation, virus passaging *in vivo*, pathogenicity studies in naïve goldfish, and temperature optima are presented. In conclusion, apparently healthy goldfish from Asia and the Middle East may transmit virulent CyHV-2 into Europe.

2. Gibel carp, *Carassius auratus gibelio*, an ancestral species of goldfish originating from China, was introduced into the Netherlands in the 17th century. Since May 2011 up to August 2016, four epizootics with a high mortality occurred in adult wild gibel carp in freshwater lakes in W-Netherlands at water temperatures of appr. 20-25°C. Clinics varied, like bleeding and pale and necrotic gills, whitish skin slime, skin haemorrhages in the head and scales, and surfacing resulting in a fast death. Internally, a pale and loose liver, a pale kidney, a haemorrhagic gut, and petechial haemorrhages in muscle and swim bladder were seen. CyHV-2 was detected in high amounts (Ct=12) by qPCR. Virus isolations on EPC and FHM cells at 15 and 20°C of the two mortalities of 2011 did not give cytopathic effect (CPE) in two passages. As gibel carp is a non-native species which seems to be very successful in the Netherlands, the CyHV-2 outbreak was believed to have had a low impact so far. In Europe, CyHV-2 in wild gibel/crucian carp was detected at least in the UK before 2010 (Way K, pers. comm. in Bergman et al., 2010), Czech Republic in 2011 (Daněk et al., 2012), and in Italy in 2011 (Fichi et al., 2013). It is not clear if CyHV-2 in wild gibel/crucian carp is emerging in Europe, as specific PCR diagnosis was introduced only recently and no monitoring has been done. However, networking, monitoring, and research on this new viral cyprinid disease is needed.

3. **Duan Hongan** : A primary cell line from allogynogenetic silver "crucian" carp *(Carassius auratus gibelio)* brain (CrCB) was established with a tissue block culture method in M199 with 10ug/mL FGF and 10ug/mL EGF. Application of stainless steel mesh with wire diameter 0.20mm and aperture

0.308mm or cover glass will facilitate the adherence of the tissue to the flask bottom. PCR fragment sequencing and alignment results showed 100% identity for the 16S and 90% for the 18s of *Carassius gibelio*. CrCB was successfully passaged to P17 with good condition. Asymptomatic goldfish with positive CyHV-2 PCR results showed symptoms and some died when water temperature was raised to about 25℃.The sick and dead fish were detected by PCR with positive results. The spleen and kidney homogenate from sick and dead fish were inoculated into the CrCB and incubated at 22℃.CPE of infected CrCB was observed at 5-6 days dpi with CyHV-2 PCR positive results. The virus isolates were passaged to passage no. 6 with steady CPE. Further characterization of CrCB and further characterization of CyHV-2 strains are needed.

References

- Bergmann S. et al. (2010) Susceptibility of koi x crucian carp and koi x goldfish hybrids to koi herpesvirus (KHV) and the development of KHV disease (KHVD). J Fish Dis 33, 267–272 (K. Way, pers. comm.)
- Daněk, T. et al., 2012. Massive mortality of Prussian carp *Carassius gibelio* in the upper Elbe basin associated with herpesviral hematopoietic necrosis (CyHV-2). Dis. Aquat. Org. 102, 87-95.
- Haenen O, Way K, Gorgoglione B, Ito T, Paley R, Bigarré L, Waltzek T (2016) Novel viral infections threatening cyprinid fish. *Bull Eur Assoc Fish Pathol* 36:11-23
- Ito T, Kurita J, and Haenen O.L.M. (submitted). Importation of CyHV-2 infected goldfish into the Netherlands through global trade. *Dis Aquat Org.*
- Wang L et al. (2012) Mass mortality caused by Cyprinid Herpesvirus 2 (CyHV-2) in Prussian carp *(Carassius gibelio)* in China. *Bull Eur Assoc Fish Pathol* 32:164-173

Questions and comments:

Uwe Fischer: *"Are cell lines susceptible for herpesvirus 3 growth?"*

Olga Haenen: *"Probably not as they are isolated from other species."*

Thomas Vesely: Samples from the Elbe (from the same case as Olga described) *"At our laboratory the isolated CyHV-2 were able to grow in normal cell lines"*

Nadav Davidovich: *"Why are the PCR positive samples not able to grow in cell lines?"*

Olga Haenen: *"I think it would be a good idea to sequence the isolates to find out if/how they are different compared to already known isolates."*

Update on Cardio Myopathy Syndrome (CMS) in Atlantic salmon

Britt Bang Jensen

Section for epidemiology, Norwegian Veterinary Institute

Postboks 750 Sentrum, 0106 Oslo

Abstract

Cardiomyopathy syndrome (CMS) is a severe cardiac disease affecting Atlantic salmon. The disease was first recognised in farmed Atlantic salmon in Norway in 1985, subsequently in farmed salmon in the Faroe Islands, Scotland and Ireland. CMS has also been described in wild Atlantic salmon in Norway. The detection and initial characterisation of piscine myocarditis virus (PMCV) in 2010 and 2011, were a significant discoveries that gave new impetus to the CMS-research.

In Norway, CMS results in reduced fish welfare, significant management related challenges and mortality in on-growing and broodfish farms. The disease thus has a significant impact on the Atlantic salmon farming industry. There is a need to gain further basic knowledge about the virus, the disease and its epidemiology, but also applied knowledge from the industry to enable the generation and implementation of effective prevention and control measures.

In 2015, the Norwegian Seafood Research Fund - FHF launched a three year research project on CMS and PMCV. 'An Epidemiological study of Cardiomyopathy Syndrome (CMS): Transmission, risk factors and disease development in Norwegian salmon farming' (CMS-Epi). The goal of the project is to increase knowledge about transmission of PMCV and factors influencing development of CMS by epidemiologic studies and a literature review. The review aims to summarise the current state of knowledge on both disease and causative agent, with special emphasis on epidemiology and disease development.

Preliminary results from the project will be presented and discussed, together with an overview of important knowledge gaps.

Questions and comments:

Niels Jørgen Olesen: *"Are there any ways of controlling the disease?"*

Britt Bang Jensen: *"The disease is all over Norway – I think that the problem will get worse in the future and that it will not be possible to control."*

Neil Ruane: *"Which method was used for the PCR?"*

Britt Bang Jensen: *"Pharmaq were partners and did this assay, so they used the published assay"*

Piscine orthoreovirus (PRV) in Europe

Niccolò Vendramin

National Veterinary Institute, Technical University of Denmark, Denmark

niven@vet.dtu.dk

Abstract

Piscine orthoreovirus – PRV have emerged as important pathogens for salmonid aquaculture worldwide.

Currently three different genotypes are proposed for this viral species.

1) PRV (PRV1) is the causative agent of Heart and Skeletal Muscle Inflammation HSMI in Atlantic salmon (*Salmo salar*)

The first cases were identified in Norway in 1999. Clinically outbreaks typically occur 5-9 months after transfer to sea water. Morbidity may be very high in affected cages, while mortality may reach 20%. This pathogen has been reported so far farmed salmon in Norway, Scotland, Faroese Islands, Iceland, France and oversea including Chile and Canada. Infection with this virus induces overexpression of IFN-related genes. The main pathological finding is the inflammatory status of the heart which is characterized by a highly cellular epicarditis. Cardiac lesions then spread to the entire myocardium developing an extensive panmyocarditis.

2-) PRV2 causing Eritrocytic Inclusion Body Syndrome (EIBS) in Coho salmon

PRV-2 is reported to induce high mortality in coho salmon (*Onchorhynchus kisutchi*) farms in Japan. The disease was first described in 1982 in juvenile chinook salmon (*O. tshawytscha*) reared in a freshwater hatchery in Washington, USA. Salmonid fish species, including chum salmon (*O. keta*), rainbow trout (*O. mykiss*), and masou salmon (*O. masou*) show different susceptibilty. Infection with the EIBS-like virus has also been reported in farmed Atlantic salmon (*Salmo salar*) in Ireland, Norway, and Scotland

3- PRV3 causing heart pathology in Rainbow trout. This virus was firstly discovered in 2014 in Norway farmed Rainbow trout. Its pathogenicity and pathogenesis was characterized in long term experimental trial in *O.mykiss* and *S.salar*, in a joint project involving DTU-VET and NVI.

The main features of these emerging pathogens in terms of epidemiology, diagnotics and pathogenesis will be discussed.

Hanne Merethe Haatveit, Øystein Wessel, Turhan Markussen, Morten Lund, Bernd Thiede, Ingvild Berg Nyman, Stine Braaen, Maria Krudtaa Dahle, and Espen Rimstad. Viral Protein Kinetics of Piscine Orthoreovirus Infection in Atlantic Salmon Blood Cells*Viruses. 2017 Mar; 9(3): 49.Published online 2017 Mar 18. doi: 10.3390/v9030049

Takano T, Nawata A, Sakai T, Matsuyama T, Ito T, Kurita J, et al. (2016) Full-Genome Sequencing and Confirmation of the Causative Agent of Erythrocytic Inclusion Body Syndrome in Coho Salmon Identifies a New Type of Piscine Orthoreovirus. PLoS ONE 11(10): e0165424. https://doi.org/10.1371/journal.pone.0165424

Olsen AB, Hjortaas M, Tengs T, Hellberg H, Johansen R (2015) First Description of a New Disease in Rainbow Trout (*Oncorhynchus mykiss* (Walbaum)) Similar to Heart and Skeletal Muscle Inflammation (HSMI) and Detection of a Gene Sequence Related to Piscine Orthoreovirus (PRV). PLoS ONE 10(7): e0131638. https://doi.org/10.1371/journal.pone.0131638

Questions and comments:

None

Red Mark Syndrome (RMS) in rainbow trout

Jacob Günther Schmidt[1], Tine Moesgaard Iburg[1], Lone Madsen[1], Mikael Strube Lenz[1], Niels Henrik Henriksen[2] and Niels Jørgen Olesen[1]

[1]National Veterinary Institute, Technical University of Denmark, Denmark

[2]The Danish Aquaculture Organisation, Denmark

jacsc@vet.dtu.dk

Abstract

Red mark syndrome (RMS) is a skin disease mainly reported to affect rainbow trout. The hallmark of the disease is large, red marks that may occur over most of the body, but commonly on the flanks. Histology shows dermatitis beginning in the stratum spongiosum and including the compactum and muscle at later stages of RMS lesion development. The disease causes little or no mortality and no substantial loss of appetite. It affects mainly large fish, and Danish fish farmers report affected fish ranging from 60-1500g. RMS has grown to be a major challenge in rainbow trout farming, as affected fish must be sold at reduced price or need to be kept until symptoms disappear. RMS has been reported from several European countries. In Denmark RMS was first observed approximately 7 years ago. Results from a 2015 questionnaire showed that it has spread to approximately one third of Danish trout farms.

Due to the increasing problems with RMS we initiated a two year project in early 2016 in order to:

Firmly establish what causes RMS
Describe RMS symptom development
Test the effect of salt, temperature, chemical water treatment and other on RMS.

An important prerequisite for these investigations was the establishment of a challenge model. Previous studies indicated that RMS was an infectious disease, and that an intracellular bacterium from the family Midichloriaceae (provisionally named Midichloria-like organism, MLO) was possibly involved. However, this could not be firmly established. Also, the prevailing opinion amongst Danish fish farmers at the time was that RMS was not infectious.

During the first year of the project we have successfully established a stable direct cohabitation model of RMS, and have transferred the disease through 5 passages of fish. Specific MLO qPCR on samples from the cohabitants showed a strong correlation of MLO 16S rDNA Ct values with RMS lesion development. Next generation sequencing of samples from the same fish further showed that MLO was the only bacterium observed to correlate with RMS lesions.

Step two in the project is underway, but the results obtained so far show a strong antibody response in the lesions. The time from start of direct cohabitation to development of early RMS symptoms is typically 500-600 degree days, which fits very well with typical time needed for development of specific antibodies in rainbow trout.

Step three will commence in the second half of 2017. Results from this will be presented on next year's EURL work shop.

Questions and comments:

Olga Haenen: *"Is there a certain rainbow trout cell line that could be used for culturing?"*

Jacob Günther Schmidt: *"No"*

Torsten Boutrup: *"Have any cell culture incubated with RMS material been kept?"*

Jacob Günther Schmidt: *"No, that is not the case."*

Torsten Boutrup: *"If it is bacteria, it is probably very slow growing."*

Anna Toffan: *"Have sampling of other organs been done?"*

Jacob Günther Schmidt: *"Yes, kidney, spleen, gill, heart, brain and liver. Sampling of other organs has not been systematic and all negative."*

SESSION III: Control and surveillance of relevant pathogens in the EU

Chair: Uwe Fischer

Carp Edema Virus Disease (CEVD) in Europe – an update

Olga Haenen

National Reference Laboratory for Fish Diseases, Wageningen Bioveterinary Research of Wageningen UR, P.O. Box 65, 8200 AB Lelystad, The Netherlands

Abstract

In this lecture, an outline of the current status of CEV detections in carp and koi *(Cyprinus carpio)* in Europe will be presented with some research results, based on the accepted alert paper for *Dis Aquat Org.*written by experts from 15 institutions in 11 European countries and USA: **Way K., O. Haenen, D. Stone, M. Adamek, S.M. Bergmann, L. Bigarré, N. Diserens, M. El-Matbouli, M.C. Gjessing, V. Jung-Schroers, E. Leguay, M. Matras, N.J. Olesen, V. Panzarin, V. Piačková, A. Toffan, N. Vendramin, T. Veselý, T. Waltzek**: **The emergence of carp edema virus (CEV) and its significance to European common carp and koi, *Cyprinus carpio*.**

Carp edema virus (CEV) disease, also known as koi sleepy disease, is caused by a poxvirus associated with outbreaks of clinical disease in koi and common carp. It was originally characterised in Japan in the 1970's. International trade in koi has led to the spread of CEV. In 1996, CEVD was reported in the USA. In 2009, the disease was first recognised in Europe.

Since its first detection in Europe, detection and diagnostic methods improved, and more EU member states reported CEV associated with disease outbreaks. So far, in Europe, Austria, Czech Republic, France, Germany, Italy, Poland, Switzerland, The Netherlands, and the UK have detected CEV.

The genome of the virus has been partly sequenced, and suggested the existence of distinct geographical populations of CEV infecting both koi and common carp. New qPCR primers successfully detected CEV DNA in formalin-fixed, paraffin-embedded archive material from investigations of unexplained carp mortalities of over 15 years ago.

Disease management and control methods, and biosecurity, good health management and disease surveillance, applied to koi herpesvirus disease, can be equally applied to CEVD. There is little chance, CEVD would be considered for notifiable disease listing. However, governments might take instigating disease control measures. Further research is needed to develop susceptible cells to isolate CEV, and to study the disease pathogenesis and epidemiology to estimate the likely impact of CEVD on European koi and common carp aquaculture and on wild carp stocks.

There is an active CEV network which organized two workshops (reports below).
Please contact Olga Haenen (olga.haenen@wur.nl) to be added to the mailing list.

- EURL (2015a) Report of Carp Edema Virus – CEV Workshop 12th – 13th January 2015 13 pp. http://www.eurl-fish.eu/Reports
- EURL (2015b) Report of Carp Edema Virus lunch meeting EAFP 2015. 9th September 2015 4pp. http://www.eurl-fish.eu/Reports

Questions and comments:

Eva Lewisch: *"Can you give us an idea on the mortality in the new cases?"*

Olga Haenen: *"We can ask Hungary?"*

Tamás Attila Juhasz: *"We found some during surveillance for KHV but it's not much really."*

Comment from the audience: *"Around 5-25%."*

Olga Haenen: *"There will be a table available on the mortality and also on coinfection with CEV."*

Update on the fish health situation in France

L. Louboutin, F. Almeras, M. Baud, L. Bigarré, J. Cabon, L. Pallandre, T. Morin

French Agency for Food, Environmental and Occupational Health & Safety, Viral Fish Pathology Unit, Université Bretagne Loire, Plouzané, France

Abstract

France is currently not totally free of regulated diseases but nevertheless contains several free disease zones and compartments. These 2 last years, Rhabdoviruses have been detected in low numbers in some farms, in the context of planned monitoring or investigations following abnormal deaths. A "ghost IHN" haunts trout farming in the Normandy region. Various isolates were detected and sequence analyses have made it possible to establish and try to explain the links between them. Recurrent outbreaks due to VHSv in Moselle (East of France) triggered important epidemiological investigations with the sampling and analysis of pikes from several ponds of the area where VHSv but also IHNv could be detected. Those various situations raise a lot of interrogations about the origin of those outbreaks. Fortunately, the situation should evolve and improve in France by the progressive establishment of a National Plan for Eradication and Surveillance for VHS and IHN, which should start before the end of the year. Not only fish farms but also ponds will be subjected to clinical visits and sampling for virological analysis, and all the positive sites should follow disinfection protocol, so that within 6 years, the metropolitan territory should be free of VHS and IHN and should achieve the category I, according to the 2006/88 European directive. In parallel, emerging diseases caused by viruses have also been regularly reported. More precisely, an increase of outbreaks due to Carp Edema Virus (CEV) has been reported since the beginning of this year, in several areas of the territory, on common carp but also on Koi. We are very vigilant on the necessity of rapidly alerting the authorities concerning the positive detections of regulated viruses but also the emerging phenomena. This monitoring work is accompanied by the continuous development and optimization of diagnostic tools, including the increasing use of NGS sequencing.

Keywords:
Sanitary situation, France, Viral diseases, VHS, IHN, CEV

Questions and comments:

Anna Toffan: *"I am curious about the pikes – were there any disease symptoms or were they completely asymptomatic?"*
Lenaig Louboutin: *"Yes, on the pond we couldn't find any hemorrhages; only the weight of the pikes was a bit high."*

A: Aquaculture in the new Animal Health Law

Knut Roenningen (presented by Niels Jørgen Olesen)

European Commission

DG SANTE – G2: Animal Health

Abstract

The new animal health law (AHL) was adopted by the European Parliament and the Council earlier this year and published in the *Official Journal* (Volume 59) 31 March. The AHL will apply from 1 April 2021 and from that date replace most of the current Union legislation on animal health, including Directive 2006/88/EC.

As AHL is a regulation, the provisions given both in the law itself and the delegated and implementing acts to be adopted by virtue of empowerment given to the Commission, will be directly binding for all parties involved, including operators, veterinarian and the Member States. With regard to aquatic animal health, the main principles in Directive 2006/88/EC are maintained in the AHL. However, some new elements have been introduced, which also will have impact on the aquatic animal health, and the most important ones are the following:

Enhanced tools for controlling diseases in populations of wild animals. Such tools will include specific transport requirements, record keeping obligations and requirements for health certification.

New criteria for listing and categorisation of diseases. All current listed diseases have to be assessed in accordance with the new criteria before the final list is adopted. The two categories "exotic" and "non-exotic" diseases will in addition be replaced by five new categories, giving more flexibility with regard to obligations for surveillance, movement restrictions and control measures.

More responsibility for disease prevention and control put on the operators such as farmers, transporters and persons responsible for slaughtering and processing plants.

The main supplementary legislation in terms of delegated and implementing acts will now within a period of three years both be drafted and adopted by the Commission. The best guarantee for a result of this process is strong involvement from the Member States.

Questions and comments:

Noted after “C: Assessment of susceptible species: Report from an OIE working group”

B: Listing of fish diseases in EU legislation

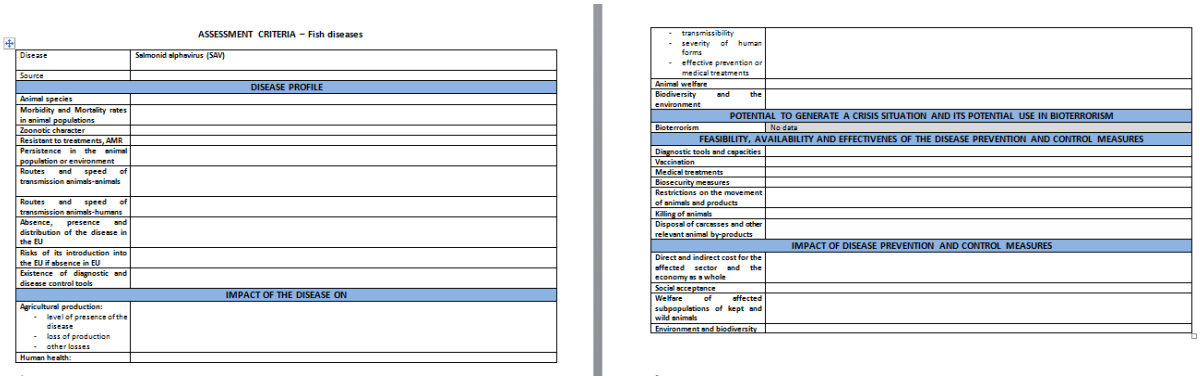
Niels Jørgen Olesen

National Veterinary Institute, Technical University of Denmark, Denmark

Abstract

An important task for implementing the new Animal Health Law will be to determine the listing of diseases in a "delegated act" comprising both aquatic and terrestrial animals.
Most likely the listing will be changed from the current list of exotic and non-exotic diseases to 5 categories:

Category a): Diseases not normally occurring in the Union
Category b): Diseases which must be controlled in al MS
Category c): Diseases subject to voluntary control in the MS
Category d): Diseases for which movement restriction measures may apply.
Category e): Diseases which shall be subject to surveillance
How the various fish diseases fit into these categories will be a matter of discussion. In order to provide the necessary background for this evaluation the EURL Fish was asked to provide updated information based on scientific studies.
As the number of diseases that should be scrutinised for the new AHL is much larger than the current 1 exotic and 4 non-exotic diseases a number of assessments are still missing but are under way. The Assessment criteria for fish diseases comprise chapters on "disease profiles", "Impact of the disease" "Potential to generate a crisis situation and its potential use in bioterrorism" and "Impact of disease prevention and control measures"



ASSESSMENT CRITERIA – Fish diseases

Disease	Salmonid alphavirus (SAV)
Source	
DISEASE PROFILE	
Animal species	
Morbidity and Mortality rates in animal populations	
Zoonotic character	
Resistant to treatments, AMR	
Persistence in the animal population or environment	
Routes and speed of transmission animals-animals	
Routes and speed of transmission animals-humans	
Absence, presence and distribution of the disease in the EU	
Risks of its introduction into the EU if absence in EU	
Existence of diagnostic and disease control tools	
IMPACT OF THE DISEASE ON	
Agricultural production: - level of presence of the disease - loss of production - other losses	
Human health:	
- transmissibility - severity of human forms - effective prevention or medical treatments	
Animal welfare	
Biodiversity and the environment	
POTENTIAL TO GENERATE A CRISIS SITUATION AND ITS POTENTIAL USE IN BIOTERRORISM	
Bioterrorism	No data
FEASIBILITY, AVAILABILITY AND EFFECTIVENES OF THE DISEASE PREVENTION AND CONTROL MEASURES	
Diagnostic tools and capacities	
Vaccination	
Medical treatments	
Biosecurity measures	
Restrictions on the movement of animals and products	
Killing of animals	
Disposal of carcasses and other relevant animal by-products	
IMPACT OF DISEASE PREVENTION AND CONTROL MEASURES	
Direct and indirect cost for the affected sector and the economy as a whole	
Social acceptance	
Welfare of affected subpopulations of kept and wild animals	
Environment and biodiversity	

Questions and comments:

Noted after "C: Assessment of susceptible species: Report from an OIE working group"

C: Assessment of susceptible species: Report from an OIE working group

Niels Jørgen Olesen

National Veterinary Institute, Technical University of Denmark, Denmark

Abstract

A new Chapter 1.5. 'Criteria for listing species as susceptible to infection with a specific pathogen' was introduced into the 2014 edition of the *Aquatic Code*. The purpose of this chapter is to provide criteria for determining which host species are listed as susceptible in Article X.X.2. of each disease specific chapter in the *Aquatic Code.* The criteria are to be applied progressively to each disease specific chapter in the *Aquatic Code*.

For species where there is some evidence of susceptibility but insufficient evidence to demonstrate susceptibility information will be included in the relevant disease-specific chapter in the *Aquatic Manual*.

Purpose

The *ad hoc* Group on susceptibility of fish species to infection with OIE listed diseases will undertake assessments for the 10 OIE listed fish diseases.

Expected outputs of the *ad hoc* Group

1. Develop a list of susceptible species for inclusion in the relevant Article X.X.2. of fish disease-specific chapters in the *Aquatic Code.*
2. Develop a list of species with incomplete evidence for susceptibility for inclusion in Section which 2.2.2. of the *Aquatic Manual.*
3. Draft a report for consideration by the Aquatic Animals Commission at their February 2017 meeting.

Questions and comments:

Uwe Fischer: *"It's important to get involved and make these decisions to be scientific and not just political."*

Olga Haenen: *"How do we get involved?"*

Uwe Fischer: *"Through the ministry."*

Niels Jørgen Olesen: *"Contact your competent authorities."*

Disease data collection in aquaculture; how to collect and interpret data

Britt Bang Jensen

Section for epidemiology, Norwegian Veterinary Institute

Postboks 750 Sentrum, 0106 Oslo

Abstract

When working with disease control and prevention, access to reliable data regarding production, transfer and disease outbreaks is imperative. Obtaining such data is a difficult task, and requires cooperation between farmers, competent authorities and laboratories/fish health services. All involved parties must understand and appreciate the purpose and benefits of data sharing.
Further, logistic systems must be in place to handle the data and analysis, and for sharing obtained information with the competent authorities and the public.
Different systems for data collection are available, but preferable are those which are automated and require little work from the suppliers of data.
When interpreting data, the source and purpose for which they were collected must always be considered. Perfect data almost never exist, but lesser data can be used, as long as biases and deficiencies are considered and communicated.

Questions and comments:

Uwe Fischer: *"How about data from companies? Will they give data to you?"*

Britt Bang Jensen: *"Yes, if you make sure it will be kept anonymous. They have to trust you. Even private laboratory results, they belong to the farmers but if you can convince them, they will give you data."*

Tablet devices in inspection and compliance visits of aquaculture facilities

Nicholas Stinton

Centre for Environment, Fisheries and Aquaculture Science (Cefas)

Barrack Road, The Nothe, Weymouth, Dorset DT4 8UB

Abstract

The Fish Health Inspectorate, Cefas, the Competent Authority for England and Wales within the UK, is responsible for the discharge of the Aquatic Animal Health England and Wales) Regulations 2009, implementing the Aquatic Animal Health Directive (Council Directive 2006/88/EC). As such inspectors from the FHI conduct regular inspections of Aquaculture Production Businesses (APB's) to undertake disease surveillance programmes, audit of farm records, collect and maintain site and business details and data collection for official industry statistics. All of which require detailing in an official record of the inspection. Ultimately this meant inspectors visiting sites with a 16 page visit form along with supporting documents covering biosecurity measures, site plans, Authorisation documents etc. The preparation of which took large amounts of time and equally so when the collected data was inputted into a database, it used large amounts of paper and there were difficulties in maintaining quality standards. Ultimately the FHI needed to move into the 21st century and so commissioned the development of a tablet based method of collecting this data and information. This method that has brought significant savings in time and efficiencies, has saved paper and ultimately improved the accuracy of the data collected but also has developed into a tool that enhances a field officers capabilities working remotely in the field and ensuring assurances of quality and security of data for the customer.

Questions and comments:

None

SESSION IV: Results from ongoing research on listed and emerging fish diseases

Chair: Thomas Wahli

Infectious Salmon Anemia (ISA) - recent development and future control

Torfinn Moldal, Maria Aamelfot, Trude Lyngstad, Lars Qviller,

Knut Falk, Edgar Brun and Brit Hjeltnes

Norwegian Veterinary Institute, P.O. Box 750 Sentrum, NO-0106 Oslo, Norway (torfinn.moldal@vetinst.no)

Abstract

Infectious Salmon Anemia (ISA) has been detected at 10-15 locations with Atlantic salmon annually in Norway during the last four years. Several local epidemics especially in the northern parts of the country give rise to concern, and phylogenetic investigations suggest horizontal transmission in many cases. ISA-virus has also been detected in rainbow trout without clinical disease.

In April 2017 the Norwegian Veterinary Institute organized a national workshop on control measures for ISA as part of an ongoing research project focusing on the impact of the putative non-virulent ISA-virus (ISAV HPR0) for ISA-outbreaks (FHF grant number 901051). ISAV HPR0 is prevalent in farmed Atlantic salmon both in Norway and the Faroese Islands, and it has been hypothesized that ISA-virus develops from ISAV HPR0 to the virulent ISAV HPRΔ. This hypothesis has gained support through a recent publication from the Faroese Islands that demonstrates transition from ISAV HPR0 to ISAV HPRΔ at a sea farm with Atlantic salmon. Similarly, both ISAV HPR0 and later ISAV HPRΔ were detected in a Norwegian hatchery that delivered smolt to four sea farms that all experienced ISA-outbreaks.

Low- and highly virulent ISAV HPRΔ display differences regarding dissemination of virus in infected Atlantic salmon. Other salmonids, Atlantic cod and corkwing wrasse among other species harbor the receptor essential for binding of ISA-virus. These findings should be followed up by experimental trials to explore the role of these species as potential carriers of virus.

Fast removal of fish from farms where ISA is detected is considered to be an important measure to prevent further spread, and intensified health control and surveillance in control areas that are established around affected farms are essential for early detection. Breeding for increased resistance may be promising, but raises questions around silent infections and carriers.

Suggested reading:

1. Aamelfot M. *et al*. Host tropism of infectious salmon anaemia virus in marine and freshwater fish species. J. Fish. Dis. 2015;38:687-694.
2. Aldrin M. *et al*. Modelling the spread of infectious salmon anaemia among salmon farms based on seaway distances between farms and genetic relationships between infectious salmon anaemia virus isolates. J. R. Soc. Interface 2011;8:1346-1356.
3. Christiansen D.H. *et al*. A low-pathogenic variant of infectious salmon anemia virus (ISAV-HPR0) is highly prevalent and causes a non-clinical transient infection in farmed Atlantic salmon (*Salmo salar* L.) in the Faroe Islands. J. Gen. Virol. 2011;92:909-918.
4. Christiansen D.H. *et al*. First field evidence of the evolution from a non-virulent HPR0 to a virulent HPR-deleted infectious salmon anaemia virus. J. Gen. Virol. 2017;98:595-606.
5. Lyngstad T.M. *et al*. Use of Molecular Epidemiology to Trace Transmission Pathways for Infectious Salmon Anaemia Virus (ISAV) in Norwegian Salmon Farming. Epidemics 2011;3:1-11.

6. Lyngstad T.M. *et al*. Low virulent infectious salmon anaemia virus (ISAV-HPR0) is prevalent and geographically structured in Norwegian salmon farming. Dis. Aquat. Org. 2012;101:197-206.
7. McBeath A. *et al*. Immersion challenge with low and highly virulent infectious salmon anaemia virus reveals different pathogenesis in Atlantic salmon, *Salmo salar* L. J. Fish Dis. 2015;38:3-15.

Questions and comments:

Debes Christiansen: *"Did you find a correlation between IPN and ISA infection?"*

Brit Hjeltnes: *"I am not sure if there is a mechanism behind the correlation (if there is one), or if it is just coincidence."*

A new case of ISA in the Faroe Islands: A diagnostic challenge?

Debes H. Christiansen

Researcher and Head of Department

National Reference Laboratory for Fish & Animal Diseases, Faroese Food and Veterinary Authority, Tórshavn, Faroe Islands

Abstract

Infectious salmon anaemia (ISA) is an important disease of farmed Atlantic salmon, *Salmo salar L.*, listed by the World Organisation for Animal Health (OIE). After first detection in Norway in 1984, epidemics have occurred in all the other major Atlantic producing countries including Canada, Chile, the Faroe Islands, Scotland and the USA, causing severe economic and production losses.

During the five-year period from spring 2000, a total of 33 ISA outbreaks were recorded in the Faroes, with all but 2 of the 25 licensed salmon farming areas being affected. Synchronised fallowing of all but one Faroese farming site with Atlantic salmon, eradication of ISA and subsequent re-establishment of the industry has provided a unique opportunity to better understand and manage the risks of re-emergence of ISA disease within this area. Practical measures employed following re-stocking have included tightening of biosecurity procedures within the industry in order to safeguard against the recognised risk of import of ISA from other affected territories, reduction in production intensity, year class separation and scheduled fallowing, vaccination against ISAV and a comprehensive screening programme for ISAV. Based on the ISAV screening program, we recently demonstrated that the non-pathogenic ISAV-HPR0 is widespread, exhibits a different tissue tropism to pathogenic variants of ISAV, shows a transient and seasonal appearance and does not result in clinical ISA (Christiansen *et al*. 2011, *J. Gen. Virol*. 92-909-918).

In July 2016, a new virulent ISAV-HPRdel strain (HPRdel) was identified by RT-qPCR through routine screening at an Atlantic salmon marine farm in the Faroe Islands. Despite intensive surveillance and testing of more than 500 fish the following five months, we were unable to confirm the presence of the HPRdel, or any clinical signs or pathological changes consistent with ISA disease. In early January 2017, the same HPRdel strain was detected by RT-qPCR, however, again without any increased mortality, clinical signs or pathological changes consistent with ISA. The following two months HPRdel prevalence increased most likely associated with bad weather and treatments for sea lice. Despite significant increase in HPRdel prevalence and testing by cell culture, haematocrit, histopathology, immunohistochemistry and clinical signs, we were not able to confirm the ISA disease diagnosis until late march, i.e. eight months post initial detection.

Here, I will present this case and the challenges we experienced from first detection of HPRdel until we finally confirmed the ISA diagnosis eight months later.

Questions and comments:

Niccoló Vendramin: *"What do you know about virus strain characteristics? In particular compared with the vaccine strain?"*

Debes Christiansen: *"They are not similar."*

Niccoló Vendramin: *"How are the health status of the fish?"*

Debes Christiansen: *"Sea lice are out of control in that particular site. It is possible to have a low pathogenicity variant that awakes when the fish are under stress by sea lice treatment. No other disease in the Faroe Islands."*

Britt Bang Jensen: *"Have you tested sensitivity for ISA in your cells? (Referring that cells can lose sensitive after a certain number of cell passages)"*

Debes Christiansen: *"We are doing that at the moment."*

Niels Jørgen Olesen: *"This study is important for the future management of disease outbreaks. What could have been done differently in this case?"*

Debes Christiansen: *"Dead fish where detected in July 2016, and the following months the detection/surveillance was based on dead fish. So, definitively take more samples from living fish in the months after the first detection."*

Niels Jørgen Olesen: *"So would you recommend following up at an early stage (immediately) or waiting until diagnosis?"*

Debes Christiansen: *"They should have wiped out the whole farm when they started with the outbreak, or definitively ring 2, because here the PCR results were strongly positive, and the whole farm two months before they did."*

Britt Bang Jensen: *"The most important is the faster removal of the fish for disease control."*

First field evidence of the evolution of a virulent infectious salmon anaemia virus (ISAV) from a non-virulent ISAV-HPR0 progenitor

Debes H. Christiansen[1*], Alastair J. A. McBeath[2], Maria Aamelfot[3], Iveta Matejusova[2], Mickael Fourrier[2], Patricia White[2], Petra E. Petersen[1], Knut Falk[3]

[1]Faroese Food and Veterinary Authority, National Reference Laboratory for Fish & Animal Diseases, Tórshavn, Faroe Islands

[2]Marine Scotland Science, Marine Laboratory, Aberdeen, Scotland

[3]Norwegian Veterinary Institute, Section for Fish Health, Oslo, Norway

Abstract

Introduction

Infectious salmon anaemia (ISA) is an important disease of farmed Atlantic salmon. The causative agent, ISA-virus (ISAV), belongs to the *Orthomyxoviridae* family. The viral genome is composed of eight single-stranded RNA-segments, encoding at least 10 proteins, including two glycoproteins: the haemagglutinin-esterase (HE) on segment 6 and the fusion protein (F) on segment 5. Differences in virulence among ISAV strains have been described in several studies. Putatively uncultivable non-virulent ISAV variants (designated ISAV-HPR0) are characterised by a HE protein carrying a full-length highly polymorphic region (HPR). All currently described pathogenic ISAV isolates associated with ISA disease have deletions in the HPR and a $Q_{266}L$ substitution, or insertion adjacent to the cleavage site in the F protein (designated ISAV-HPRdel). In January 2014, a new ISAV-HPRdel strain (HPRdel) was identified through routine screening at an Atlantic salmon marine farm in the Faroe Islands. The purpose of this study was (i) to investigate whether the HPRdel virus originated from any of the HPR0 variants known to circulate in Faroese aquaculture at that time and (ii) to characterize the virulence of the HPRdel virus.

Methodology

The study population included seven epidemiologically linked marine farms within a management area which were supplied with stocks from seven freshwater smolt farms. A total of 4537 fish were screened by ISAV specific real-time RT-PCR and partial segment 6 of all 520 ISAV positive samples were sequenced to determine HPR-subtypes. The ORF of all eight segments from a linked freshwater HPR0 and the marine HPRdel were sequenced to confirm their identities. The virulence of the HPRdel variant was determined using an *in vivo* immersion challenge and *in vitro* experiments.

Results

Here, we provide the first evidence of genetic and functional evolution from a non-virulent ISAV-HPR0 to a low-virulent ISAV-HPRdel in a Faroese Atlantic salmon marine farm. The HPRdel infection was not associated with specific clinical signs of ISA and was confined to a single net-pen, while various HPR0-subtypes were found circulating in most epidemiologically linked marine and freshwater farms. Sequence analysis of all eight segments revealed that the HPRdel virus was identical, apart from a substitution in the F gene ($Q_{266}L$) and a deletion in the HE gene, to the HPR0 variant from a freshwater farm, which supplied smolts exclusively to the HPRdel positive net-pen. In an infection challenge the HPRdel virus induced a systemic infection in Atlantic salmon associated with a low mortality and mild clinical signs confirming its low pathogenicity.

Conclusion
Our results demonstrate that mutations in the F protein and deletions in HPR of the HE protein represent important first-step mutations and a minimum requirement for ISAV to gain virulence and to switch cell tropism from a localized epithelial infection to a systemic endotheliotropic infection. This suggests that ISAV-HPR0 represents a reservoir and risk factor for the emergence of ISA disease.

Questions and comments:

Britt Bang Jensen*: "Time of infection effects pathogenicity. If you have a recirculating system, can this system promote infection, so the recommendation is to reduce the time in the farms and increase the time at sea?"*

Torsten Boutrup: *"Yes, but in RAS system you have the possibility of using other tools to control some of the factors, so the recommendation would be to use the RAS in good way and not only to increase the production volume, but use it to reduce stress in the fish."*

Niels Jørgen Olesen: *"There is a whole range of deletions in the ISA isolates, can you correlate deletions with a change in virulence?"*

Debes Christiansen: *"No. Identical deletions do not correlate with virulence, so there must be other virulence markers than only the deletion."*

In the search for virulence markers of Viral Hemorrhagic Septicemia Virus (VHSV)

Anna Luiza Farias Alencar[1*], Argelia Cuenca Navarro[1], Thomas Bruun Rasmussen[1], Yannick Blanchard[2], Michel Bremont[3], Niels Jørgen Olesen[1]

[1] Danmarks Tekniske Universitet, National Veterinary Institute, Frederiksberg, Denmark; [2] Agence Nationale Sécurité Sanitaire Alimentaire Nationale, Ploufragan, France; [3] Institut national de la recherche agronomique, Jouy-en-Josas, France.

alfal@vet.dtu.dk

Abstract

Viral Hemorrhagic Septicemia virus (VHSV) is responsible for high morbidity and mass mortality in marine and freshwater fish with high economic losses in European rainbow trout farming. Significant variations in virulence to rainbow trout are observed among different VHSV isolates. It is of need for the fish farming industry to have tools to discriminate between these virulent and non-virulent VHSV isolates. Twelve different VHSV isolates from the DTU-Vet VHSV repository were selected based on their virulence pattern and genotype, and were propagated in cell culture and plaque cloned. Thirteen viral clones were collected, propagated and whole genome sequenced and in addition subjected to an experimental infection trial by bath in rainbow trout, in order to assess their virulence in this species. The morbidity in the infection trial varied from 2,7% (VHSV isolate Fin Ka 423/00) to 99,7% (VHSV isolate DK 203490) among isolates and also showed that genotypes Ie and Ic had most differences in virulence among isolates in the same genotype. These results indicate that while these isolates are genetically close, they are different when it comes to virulence and therefore whole genome sequences alignment analysis was carried out in order to identify possible virulence markers. By site directed mutagenesis using a low virulent VHSV isolate as a backbone these putative markers will be assessed by the generation of recombinant viruses and then tested in *in-vivo* trials with Rainbow trout. This study is part of the anihwa ERA-Net project 023 NOVIMARK with financial support from Innovation Fund Denmark.

Questions and comments:

None

Recurrent unexplained mortalities in warm water fish species; emergence of new Tilapia virus and epidemiological approach for surveillance

Nadav Davidovich

Israeli Veterinary Services, P.O. Box 12, Bet Dagan 5025001, Israel

Abstract

Tilapia is one of the most common cultivated fish species worldwide. In 2012, the annual global production of Nile tilapia (*Oreochromis niloticus*) was 3.1 milion tones. Over 90% of farmed Tilapia are produced in developing countries, mainly in Asia. In Israel, the leading species in the inland aquaculture is a Tilapia hybrid (*Oreochromis niloticus* X *O. aureus*), the total production in 2016 reached 7.5 thousand tones. Tilapia are popular fish for culture due to of their hardiness, breeding success, short grow-out cycles, easy handling, appealing flavor and tolerance to a wide range of environmental factors, including fresh and brackish water.

Although Tilapia are considered relatively resistant to diseases, under intensive rearing they are susceptible to common aquatic pathogens: viruses, bacteria, fungi, water molds and parasites. Despite the global dispersion of Tilapia, only few viruses have been described from Tilapia and none has been proven to be a serious threat to the industry. RNA viruses that were isolated from tilapia included *Aquabirnavirus* and *Betanodavirus*. The DNA viruses which have been described in Tilapia were belonging to the family *Iridoviridae* (*Megalocytivirus*, *Lymphocystivirus*, and *Ranavirus*) and *Herpesvirus*. In 2014, a novel RNA virus termed Tilapia Lake Virus (TiLV) was described by Eyngor *et al*. The virus was isolated in Israel as a result of an investigation into Tilapia mortalities in wild and farmed stocks. The virus was also associated with mortalities of farmed Tilapia in Ecuador and Columbia. The authors described more than 80% mortality in a cohabitation trail of healthy and infected fish. In 2017, the virus was also described in farmed Tilapia in Egypt and Thailand. In an epidemiological surveys conducted by Fathi *et al.*, 2017, following unexplained Tilapia mortalities, the average mortality rate related to TiLV was estimated as 9.2%.

Information regarding the new virus, such as, infectivity to different Tilapia species, attack rate, fatality rate and clinical manifestation are scarce. In addition, environmental factors and the complexity of open system aquaculture make it challenging to estimate the true impact of the new agent. Aiming to gain more information about Tilapia mortalities and survival rates in the different production stages, The Israeli Veterinary Services and the Koret School of Veterinary Medicine designed an epidemiological retrospective survey. The survey will try to determine factors influencing low survival rates and overall mortalities including relative importance of TiLV.

Questions and comments:

Olga Haenen: *"Which cell line do you use?"*
Nadav Davidovich: *"E-11 cell line."*

Barcoding of fish cell lines - the origin of cell lines is not always what we believe

Argelia Cuenca Navarro and Niels Jørgen Olesen

National Veterinary Institute, Technical University of Denmark, Copenhagen, Denmark

Abstract

A Barcode sequence is a short nucleotide sequence from a standard genetic locus for use in species identification. For animals, the first 650 base pair at the 5' end of the mitochondrial cytochrome oxidase subunit I (COI) gene are used as standard genetic locus, although other genes have also been proposed. At the EURL for fish diseases, we have successfully barcoded a number of cell lines from our repository and generated a standard operational procedure to do so. The main idea is to use cell barcodes to identify the origin of our cell lines and to detect possible cross contamination in our cell library. As one of the tasks of the European Reference Laboratory for fish diseases is to supply cell lines all over the world, barcoding will be implemented as an additional quality assessment in the import/export of cell lines among countries and laboratories.

Among our preliminary results, we have detected a number of cell lines which barcode does not correspond with the species of which they originated (e.g. EPC, CCO, ASK). We are in the process to detect if a species misidentification would happened when the cell line was created (as may be the case of EPC), or a problem occurred later as crossing over contamination.

Cell line	Name	Expected origin	Latin	De facto origin	
EPC*	*Epithelioma Papullosum Carpio*	Common carp	*Cyprinus carpio*	*Pimephales promelas*	Fat Head Minnow
BF-2	Bluegill Fry	Bluegill	*Lepomis macrochirus*	*Lepomis cyanellus or Lepomis macrochirus*	Green sunfish / bluegill
CHSE-214	Chinook Salmon Embryo	Chinook Salmon	*Oncorhynchus tshawytscha*	*Oncorhynchus tshawytscha*	Chinook Salmon
RTG-2	Rainbow trout gonad	Rainbow trout	*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	Rainbow trout
FHM	Fat Head Minnow	Fat Head Minnow	*Pimephales promelas*	*Pimephales promelas*	Fat Head Minnow
CCO	Channel Catfish Ovary	Channel Catfish	*Ictalurus punctatus*	*Ameiurus nebulosus*	Brown bullhead
EK-1	Eel Kidney	Pacific eel.	*Anguilla anguilla or Anguilla obscura*	*Anguilla japonica*	Japanese eel
ASK	Atlantic Salmon Kidney	Atlantic Salmon	*Salmo salar*	*Oncorhynchus mykiss*	Rainbow trout
CCB	Common Carp Brain	Common Carp	*Cyprinus carpio*	*Cyprinus carpio*	Common Carp
SBL	Sea Bass Lymphoid	European seabass	*Dicentrarchus labrax*	*Oncorhynchus tshawytscha*	Chinook Salmon
WSSK	White Sturgeon SKin-1	White sturgeon	*Acipenser transmontanus*	*Acipenser transmontanus*	white sturgeon

Questions and comments:

Olga Haenen: *"Originally EK 1 cell are from Japan (also in the publication)."*

Ellen Ariel: *"We have the same, that EPC is FHM"*

Debes Christiansen: *"What is the difference in BOLD from Rainbow trout to Atlantic salmon?"*

Argelia Cuenca Navarro: *"should be 90% similarity in two genes."*

Richard Paley: *"Will communicate in this findings with ATCC?"*

Niels Jørgen Olesen: *"We will and it is given."*

Anna Toffan: *"Not all will be available on ATCC."*

Validation of Viral Haemorrhagic Septicaemia (VHS) virus conventional RT-PCR

Hyoung Jun Kim[1*] and Niels Jørgen Olesen[2]

[1]National Fishery Products Quality Management Service, Busan, Korea

[2]Technical University of Denmark, National Veterinary Institute, EU Reference Laboratory for Fish Diseases, OIE Reference Laboratory for VHS, Kgs. Lyngby, Denmark

Abstract

Conventional PCR is regularly used for detection and genotyping of pathogens. However, we found a low sensitivity for detection of VHSV IVa isolates using the conventional RT-PCR described in the OIE aquatic manual (VN primer set). In addition, non-specific bands with fish cell lines were often observed when using the OIE RT-PCR. Thus, a novel conventional RT-PCR (3F2R method) has been developed and announced at the 20th annual workshop of the National Reference Laboratories for fish diseases.

The 3F2R method showed the same sensitivity and specificity as cell culture and real-time RT-PCR. No specific responses were observed in heterologous viruses and normal fish cell lines and the 3F2R method was subsequently tested on 80 VHSV isolates representing a worldwide collection of all known genotypes and subtypes, where it produced clear and unique amplicons for all 80 isolates.

In this study, we clearly stated why the VHSV RT-PCR in the current OIE manual had low sensitivity to VHSV genotype Iva and we confirmed the specificity of the 3F2R method on organ materials from rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*) and olive flounder (*Paralichthys olivaceus*) infected with a range of VHSV isolates.

For assessment of the reproducibility of the 3F2R method, an inter-laboratory proficiency test was conducted in nine laboratories. Ten samples were prepared on FTA cards including six VHSV (Genotype I, Ib, II, III, IVa and IVb), three heterologous viruses (IPNV, HRV, and IHNV) and one normal cell culture supernatant. The viral supernatants were dropped on FTA cards, and sent to each laboratory together with a detailed SOP and information on the 3F2R primers.

Among the nine laboratories, one laboratory did not detect all VHSV isolates using 3F2R primer, EZ-1 RNA tissue mini kit and EZ-1 BioRobot and a two step RT-PCR using MMLV and Go-Taq (Promega). It seems that the RNA extraction from the FTA cards was not conducted smoothly. The PCR results from the other eight laboratories were, however, successful using the 3F2R primer set. Several different RNA extraction kits and conventional RT-PCR kits were used. Therefore, we confirmed the robustness and reproducibility of the 3F2R RT-PCR and the usefulness of FTA cards for VHSV gene detection.

Finally, we suggest that the 3F2R primer set shall replace the current primer set recommended in the OIE manual for detection of VHSV.

Questions and comments:

None

New Viral Haemorrhagic Septicaemia (VHS) virus subtype in Europe

Argelia Cuenca[1], Niccoló Vendramin[1], Heiða Sigurðardóttir[2], Niels Jørgen Olesen[1], Sigríður Guðmundsdóttir[2]

[1]National Veterinary Institute, Technical University of Denmark, Copenhagen, Denmark; [2]Institute for Experimental Pathology, University of Iceland, Reykjavik, Iceland

Abstract

Viral haemorrhagic septicaemia virus (VHSV), a ssRNA virus from the family Rhabdoviridae, has been isolated from more than 90 fish species in freshwater and marine environment in the northern hemisphere. In 2015, VHSV was isolated from lumpfish (*Cyclopterus lumpus*) caught in Breiðafjörður Bay on the west coast of Iceland. This constitutes the first report of VHSV in the country. Moreover, this is the first time that VHSV is found in lumpfish, and genotype IV appear in European waters. Genetic characterization showed that this new isolate is genetically different from other European VHSV isolates, and more similar to isolates from the Atlantic coast of Canada and USA. Indeed, phylogenetic analyses based on the full-length sequences of the N, G and NV genes placed the Icelandic isolate as closely related to genotype IV isolates from North American and Asia, but without being part of any of the currently recognized genotype IV subgroups. In this work, we propose the creation of a genotype IVd to place the lumpfish VHSV isolate from Iceland.

Questions and comments:

Niels Jørgen Olesen: *"It is important to screen lumpfish in Norway for VHSV"*

Neil Ruane: *"What were the consequences in Iceland?"*

Sigríður Guðmundsdóttir: *"Stamping out the facility and Chile stopped import of Icelandic Atlantic salmon roe for 6 months."*

Sample preservation for PCR

Argelia Cuenca, Tine Iburg, Niccoló Vendramin, Troels Rundqvist, Christina Desler, Teena Klinge, Niels Jørgen Olesen.

National Veterinary Institute, Technical University of Denmark, Copenhagen, Denmark

Abstract

It has been suggested that freezing and thawing cycles can strongly affect detection of certain RNA fish viruses. In particular, Ct values seem to increase considerably after only one round of freezing and thawing of piscine orthoreoviruses. In certain occasions, no all samples sent to a diagnostic laboratory can be tested immediately, so an alternative to freezing samples for short/medium term storage need to be found. We evaluate using a mix of transport media (MEM) and lysis buffer for sample storage up to four weeks. Both RNA and DNA viruses were tested, using either spiked organ supernatant or positive tissue. After a four weeks period, samples were tested by qPCR. Results indicate that storage in MEM and lysis buffer do not affect Ct values after such period of time. With this in mind, we are expecting to perform similar tests for long-term sample storage.

Questions and comments:

Prapas Athanasios: *"How much lysis buffer did you use?"*

Argelia Cuenca Navarro: *"400 microliters of lysis and 400 of tissue"*

Anna Toffan: *"Did you use RNA later?"*

Argelia Cuenca Navarro: *"Normally we use RNA later, but not in this case, since we were pooling different organs"*

Richard Paley: *"Did you compare freezing and thawing sample?"*

Niccolò Vendramin*: "No, it does not work to freeze PRV"*

Uwe Fischer: *"We normally preserve the organs directly in lysis for gene expression analysis"*

Olga Haenen: *"Does anyone use biofreezer?"*

Audience: *"No"*

Sigríður Guðmundsdóttir: *"What about RNA?"*

Argelia Cuenca Navarro: *"We freeze controls in -80 Celsius."*

Use of FTA cards in fish diagnostics

Argelia Cuenca, Tine Iburg, Troels Rundqvist, Niels Jørgen Olesen

National Veterinary Institute, Technical University of Denmark, Copenhagen, Denmark

Abstract

One of the tasks of the European Reference Laboratory for fish diseases is to supply reagents such as reference pathogens strains all over the world. A continuous challenge in this task is to maintain suitable transport conditions and fulfill legal demands for shipping live viruses abroad. In a framework where more and more molecular techniques are advancing in the field of fish diagnostics, not requiring live pathogen to be tested, we have investigated the use of FTA cards to ship inactivated viruses and assess their preservation after shipment. In recent years, Whatman® FTA® card have been used as an alternative to transport and preserve biological samples, and the possibility to use for safe transport of virus has been tested in a number of disease outbreaks (e.g., avian influenza, rabies, infectious bronchitis, among others). This method has two main advantages: first, it removes the need to preserve and transport samples in cold conditions, and second, it allows safe handling of infectious samples, as viruses are inactivated once placed in the FTA® card.

Here we evaluate the use of FTA® cards for storage of inactivated virus samples. In particular, we are interested in knowing for how long time the virus can be stored in FTA cards and still detected with routine procedures. As this probably varies among different viruses and storage conditions, we evaluated a representative of a DNA virus (KHV) and one of a RNA virus (VHSV), with FTA cards stored at room temperature and at 37 °C.

In addition, different protocols for elution and recovery of DNA and RNA viruses from FTA cards were evaluated.

Questions and comments:

Anna Toffan: *"What about sequencing? We observed degradation in the sequences."*

Sigríður Guðmundsdóttir: *"We tried for BKD, there are two types. One is for conventional PCR and the other for qPCR (FTA elute)."*

Argelia Cuenca Navarro: *"We use the classical one."*

Uwe Fischer: *"Do you think is it only a dilution effect you observe from first day?"*

Argelia Cuenca Navarro*: "No, the protocol needs to be optimized."*

Niels Jørgen Olesen: *"What is the difference between the two options?"*

Argelia Cuenca Navarro: *"We have a specific kit for virus RNA."*

Prapas Athanasios: *"Are both kits Qiagen?"*

Argelia Cuenca Navarro: *"Yes."*

Richard Paley: *"We used them long time ago, there were issue in replication."*

Anna Toffan*: "We receive them from time to time. We use the whole card on TE ebuffer overnight and then purify."*

SESSION V: Update from the EURL

Chair: Niels Jørgen Olesen

Results of the proficiency test, PT1 and PT2, 2016

Niccoló Vendramin[1], Teena Vendel Klinge, Argelia Cuenca and Niels Jørgen Olesen

[1] EU Reference Laboratory for Fish Diseases,

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

Abstract

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

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 for 2015, also in 2016 the EURL decided that the panel of pathogens to be investigated should include Salmonid Alphavirus (SAV). Since SAV is not a listed disease in the European legislation, all participants were free to decide if they would be testing for SAV or not. Each participant was asked to declare whether they would test or not. The EURL would then take care of calculating the score accordingly.

45 laboratories participated in PT1 while 43 participated in PT2.

The tests were sent from the EURL in the end of September 2016.

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 Commission Decision 2015-1554.

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

The number of National Reference Laboratories (NRLs) participating in PT1 and PT2 was 43.

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.

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
60% of parcels were delivered by the shipping companies within 1 day after submission and 86% was delivered within 1 week. The remaining six parcels took longer for delivery primarily due to border controls, the maximum time of shipment was 21 days.

This year ECV was included in the Proficiency test. 37 participants provided the correct identification, 1 laboratory identified correctly the isolate but contaminated the ampoule content.

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

This year pike fry rhabdovirus was included in ampoule I. This virus has generated some challenges to the participants due to its antigenic similarity with SVCV, however the increase implementation of biomolecular techniques has allowed 17 laboratories to identify it correctly and other 17 were able to rule out the presence of VHSV,IHNV,IPNV,SVCV and ranavirus. The scoring system has been adjusted accordingly.

Overall 31 out of 45 participants scored 100% success rate and 8 more than 90%.
It has been a concern that few laboratories has identified the correct virus but not in the right ampoule, meaning that some mistake in traceability of the ampoules during the working flow procedure has occurred. Another critical points that has emerged, is the contamination of ampoule contents. These points will be assessed directly with the single participants that has underperformed.

PT2 conclusion
After the positive experience in 2015, the EURL decided to include SAV in the panel of viruses included in PT2. Considering that 33 laboratories participated in 2015 (of which 32 correctly identified SAV in ampoule VII) this was regarded as a proper initiative that strengthen the diagnostic capacities of the NRLs in detecting emerging pathogens, and it will be included in the coming years as well.
37 laboratories participated in PT2 testing for SAV and all of the 37 correctly identified the virus in Ampoule VI.
42 out of 43 laboratories correctly identified the ISA virus in ampoule VII.
Out of 43 participants, 2 did not test for KHV and 1 did not identify the virus in Ampoule VIII, the other 40 correctly detected KHV in ampoule VIII.
It is an appreciated matter of fact that many laboratories are putting efforts in performing genetic characterization of the isolates through sequence analysis, as it is important that laboratories can discriminate between isolates as e.g. pathogenic ISAV strains with deletions in the HPR region and HPR0 strains, especially after the delisting of ISAV HPR0 (Commission Implementing Directive 2014/22/EU).

Questions and comments:

Nikolaj Gedsted Andersen: *"How many laboratories make errors of contamination/mix up of samples?"*

Niccoló Vendramin: *"I don't have the exact number, but many."*

Marine Baud: *"Regarding EHNV, should we make the sequence or it is enough to distinguish both variants by conventional PCR? Both variants have different size (deleted vs. non-deleted)."*

Niels Jørgen Olesen: *"I am not sure, in any case the protocol needs to be validated."*

Niccoló Vendramin: *"We need a large repertoire of EHNV virus for validating conventional PCR, maybe Ellen Ariel could help with that?"*

Ellen Ariel: *"Sure."*

Niels Jørgen Olesen*: "It would also need to be published."*

Niccoló Vendramin: *"In this PT and in the future we will be focusing more on sequencing and genotyping, as the reference labs need to be able to do virus characterization. It is important to test the strength of sequencing techniques, as we are still detecting large number of errors there."*

EURL Training Courses. Topics and organization for courses 2017

Nikolaj Gedsted Andersen and Tine Moesgaard Iburg

National Veterinary Institute, Technical University of Denmark,

Kemitorvet, building 202, 2800 Kgs. Lyngby

ngaan@vet.dtu.dk and timi@vet.dtu.dk

Abstract

For 2017, the EURL for fish diseases will organize two training courses.
The courses available are:

- **Methods for implementation of surveillance procedures for listed fish diseases**
  The course will be held in week 41 from Monday the 9th to Friday the 13th of October

- **Introduction to histopathology in fish diseases**
  The course will be held in week 42 from Monday the 17th to Thursday the 20th of October

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

Questions and comments:

Tuija Kantala: *"Should you consider making a course in sequencing analysis and NGS?"*

Niels Jørgen Olesen*: "Bioinformatics in fish diseases and in other fields is the same, and the course needs to be specific for fish diseases. This course would have to be more general, and there are many of those already."*

From the audience*: "There could be a course in non-lethal testing (KHV). It has been used a lot before with good results."*

Niels Jørgen Olesen: *"E-mail ideas to Nikolaj. We also have possibilities within Aquaexcel."*

Olga Haenen: *"Maybe a zebra fish disease course? It is widely used in hospitals."*

Niels Jørgen Olesen: *"Do not think that we could use EURL money for that."*

Niels Jørgen Olesen: *"Maybe we could think in a Tilapia disease course. In addition, as a reference lab we could diversify and start providing virus panels for validations, etc."*

EURL activities in 2016

Niels Jørgen Olesen

EU Reference Laboratory for Fish Diseases,

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

Abstract

The National Veterinary Institute, Technical University of Denmark (DTU-VET) is appointed as the European Union Reference Laboratory for Fish Diseases (EURL), in accordance with the Commission Implementing Regulation (EU) No 135/2013 of 18 February 2013, the notification of grant decision for an action regarding the EU Reference Laboratory for Fish Diseases – SI2.725290 and the corresponding grand decision (Ref. Ares(2016)854560 - 18/02/2016) as regards the Union financial aid for the year 2016 and 2017 to the EURL Fish Diseases

The duties of the EURL are described in Council Directive 2006/88/EC of 24 October 2006 (Annex VI). The duties mainly concern fish diseases listed as exotic diseases: epizootic haematopoietic necrosis (EHN); and fish diseases listed as non-exotic diseases: infectious salmon anaemia (ISA), viral haemorrhagic septicaemia (VHS), infectious haematopoietic necrosis (IHN) and koi herpes virus disease (KHVD). This report follows the format of the work programme adopted for the EURL for 2016 and 2017, describing activities and sub-activities and the status of on-going projects. The list of functions and duties of the EURL follows this introduction.

The 20th Annual Meeting of the National Reference Laboratories for Fish Diseases was held in Copenhagen, Denmark, May 31st – June 1st at the premises of the Veterinary Institute. A total of 65 participants from 33 countries attended over the two days period. There were five sessions with a total of 29 presentations, 3 of which were given by invited speakers, a working group session and a round table discussion.

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

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

Another important focus area was the development, implementation and validation of diagnostic tools for identification of the listed and emerging diseases and their accreditation. One outcome of these efforts was the implementation of a real time RT-PCR for detection of Calicivirus, a real-time PCR

for detection of salmon pox virus and *Renibacterium samoninarum*, and optimization and validation of a real time RT-PCR for surveillance and diagnosis of IHNV.

During 2016, resources were again used to collate data on surveillance, health categorisation, and diagnostics in EU, this year in an amended and more simplified form; to identify and characterise selected virus isolates; to type, store and update a library of listed virus isolates; to develop, update and maintain the database containing information on fish pathogens (www.fishpathogens.eu); to supply reference materials to NRLs; to provide training courses in laboratory diagnosis; to update the EURL webpage (www.eurl-fish.eu); and finally to attend international meetings and conferences.

In 2016 our molecular biologist since 2012, Dr. Susie Sommer Mikkelsen, decided to leave her position for a position back in Jutland. She is now replaced by Dr Argelia Cuenca Navarro with a strong background in molecular biology and bioinformatics. In addition our coordinator since 2012 DVM Niccolò Vendramin obtained a 2 year sabbatical leave from the EURL in order to focus on finalizing his PhD study. He is replaced by Dr. Nikolaj Gedsted Andersen with a significant scientific background

Questions and comments:

None

EURL workplan for 2017; ideas and plans

Niels Jørgen Olesen

EU Reference Laboratory for Fish Diseases,

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

Abstract

	Description	Objectives	Expected outputs
1. Coordination and training			
1-1	**Annual workshop**	Organize and prepare for the 21st Annual Workshops for the National Reference Laboratories for Fish Diseases (NRLs) in 2017	To be held during the final week of May in 2017
1-2	**Annual workshop report**	Produce a technical and financial report from the Annual Workshops in 2017.	To be finalized and submitted August 2017
1-3	**Survey& diagnosis**	Collect and report data on the fish disease situation in EU, including all the listed non-exotic fish diseases given in Council Directive 2006/88/EC Annex IV Part 2.	A questionnaire will be submitted in January 2017 and data collated for the Annual Workshop in May.
1-4	**Training**	Facilitate and provide training in laboratory diagnosis. The yearly training courses in methods used for diagnosis of fish diseases will be offered to the EURL laboratory facilities. The courses will primarily be for training of staff from NRLs and the content will depend on request from participants.	Training courses are provided fall October-November 2017; two courses each year of 3-5 days each with expected 15 participants are foreseen.

1-5	**Scientific working group**	Organize specific scientific meeting collating international experts to assess and provide recommendations on management and control of emerging diseases problems in EU.	One meeting gathering 5 to 7 international experts will be held at our premises or on spot according to disease case in 2017. Scientific reports and recommendations will be delivered afterwards to relevant stakeholders.

2. Proficiency test			
2-1	**Proficiency tests**	Prepare the Annual Inter-laboratory Proficiency Tests year 2017 for the NRLs. The tests will include VHSV, IHNV, EHNV, ISAV and KHV and will also address other common viral pathogens in fish farming (IPNV, SVCV, SAV, Ranaviruses etc)	To be shipped fall 2017, respectively (tentatively mid-September)
2-2	**PT reports**	Collate and analyse information gained from the Inter-laboratory Proficiency Tests	Report for the proficiency test 2016 was submitted February 2017
3. Reagents and products			
3-1	**Supply of Reagents**	Supply reference reagents to the NRLs in Member States.	Reagents as monoclonal antibodies, rabbit antisera, pathogen isolates or cell cultures are expected to be send to approx. 15 laboratories in 2017,
3-2	**Production of reagents**	Production of diagnostic reagents against selected pathogens when necessary	Diagnostic reagents (i.e. polyclonal antibodies raised in rabbit, monoclonal antibodies from stored hybridoma cells or In Situ Hybridization -ISH probes) will be produced according to demand
3-3	**Pathogen library**	Update and maintain a library of isolates of infectious salmon anaemia virus (ISAV), Viral Haemorrhagic Septicaemia virus (VHSV) and Infectious Hematopoietic Necrosis virus (IHNV), Koi Herpes virus (KHV) and Enzootic Hematopoietic Necrosis virus (EHNV) and other relevant putative emerging fish pathogens.	The library will be updated with 10 to 20 pathogen isolates

4. Scientific advice and activities			
4-1	**Webpage**	Update the webpage for the EURL, www.eurl-fish.eu	Keep the webpage constantly updated, uploading relevant material (e.g. AW report, AW presentations, Training course report etc.,)
4-2	**Diagnostic manuals**	Update the diagnostic manuals for VHS, IHN, ISA, KHV disease, EHN on the EURL web page.	The diagnostic manual for sampling and detection of listed non-exotic diseases was finally adopted in 2015. But as the diagnostic procedures for identification and surveillance of the listed diseases is rapidly evolving new procedures will be assessed and validated for inclusion in the first revision of the diagnostic manual.
4-3	**FishRefLabNet**	Maintain and further develop the interactive network with the NRLs, FishRefLabNet, in order to promote a proactive data sharing and communication with and between reference laboratories in the Member States.	The webpage and mailing list based platform for communication and data sharing will be continued with periodical updates sent to all members that subscribed.
4-4	**Pathogen characterization**	Identify and characterize selected isolates of listed viruses (pathogenicity testing in-vivo and in-vitro, serological and genetic characterization).	The EURL receive every year strains and samples for corroboration of diagnostic results in EU Member states. Regularly these strains must be characterized properly as an emergency response to avoid unwanted spreading of new pathogens in EU
4-5	**www.fishpathogens.eu**	Update and expand www.fishpathogens.eu with more pathogens.	The database is a valuable tool for virus characterisation and molecular epidemiology. The more isolates included the stronger the tool. New databases on other listed and emerging pathogens are in the pipeline such as a database on SAV (pancreas disease and sleeping disease viruses). At least 50 new isolates are envisaged to be included and 1 new database opened in 2016 and 2017.
4-6	**Molecular epidemiology**	Perform molecular epidemiological analysis to improve knowledge on diseases spreading mechanisms of the listed viral fish pathogens.	A study involving isolates from several Continental European countries is envisaged.

4-7	**Real-time PCR**	Assessment and standardisation of real-time PCR tests for the diagnosis, identification and typing of emerging and the listed non-exotic and exotic fish diseases.	Real-time PCR is a highly sensitive and specific tool for diagnosis and surveillance of a number of listed pathogens. Published and non-published methods will be assessed in our premises in order to offer validated protocols for the NRL´s

4-8	**Emerging diseases**	In collaboration with specialised experts WW to review selected emerging fish diseases in Europe and assess their potential listing as exotic or non-exotic diseases	Due to increased international trade of fish focus will be given to emerging diseases and rapid response. An assessment of risk for contracting and spreading emerging and re-emerging diseases in EU will be enforced in 2016 and 2017 (e.g. CEV – Koi sleepy disease; Piscine orthoreovirus infections in Rainbow trout and salmon, RLO-Rickettsia like organism in Sea bass, new high virulent strains of IHNV etc.)
4-9	**Producing virtual teaching material (e-learning)**	Preparing virtual guidelines for conducting proficiency tests. For sampling and shipment of material for laboratory examination; and for receipt and processing fish tissue material for virology (inoculation on cell cultures and for PCR analysis) and histopathology	Set up tools for producing e-tutorials in-house. One tutorial on Dissection of fish for sampling for histopathology will be produced.
4-10	**Molecular characterization of fish cell lines**	Perform molecular analysis to “bar-code” and certify cell lines routinely used for viral diagnostics.	Misclassification of cell culture has been an issue constantly affecting cell culture work in terrestrial animals (including humans). In order to guarantee uniform and certified cell lines, genetic characterization and certification of relevant fish cell lines (i.e. EPC, BF-2) will be implemented

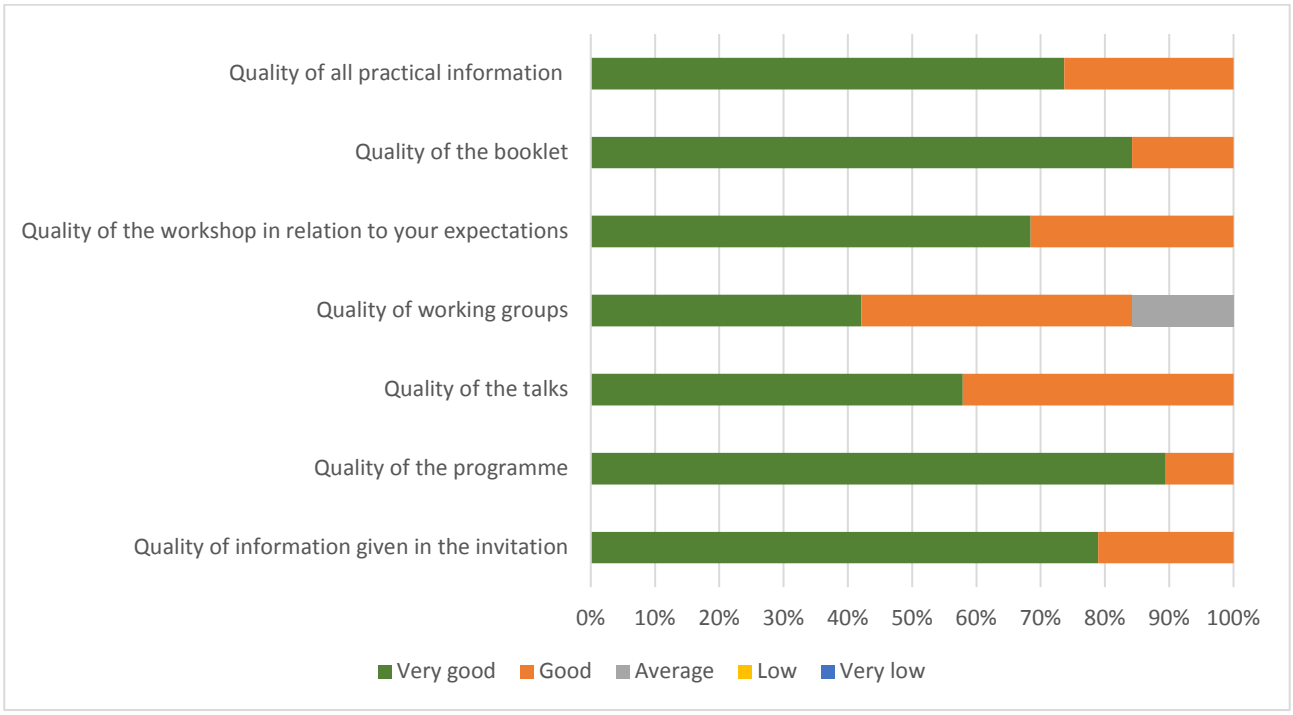
5. Missions and international meetings			
5-1	**Missions**	Organizing missions to relevant laboratories in EU and in third countries. Missions will focus on NRLs where on-site communication would be beneficial. As collaboration with NRLs in 3rd countries from where EU is importing large amount of fish products is increasing, missions to these, e.g. China and Korea is foreseen	1-2 missions will be conducted each year. The laboratories to visit will be appointed in order to strengthen collaboration in the NRL network. (e.g. Spain, France, Korea, Iran etc…)ni
5-2	**International meetings**	Attending missions, international meetings and conferences in order to be updated on emerging and listed exotic and non-exotic fish diseases.	The EURL expect to participate in 2 to 3 international conferences each year.

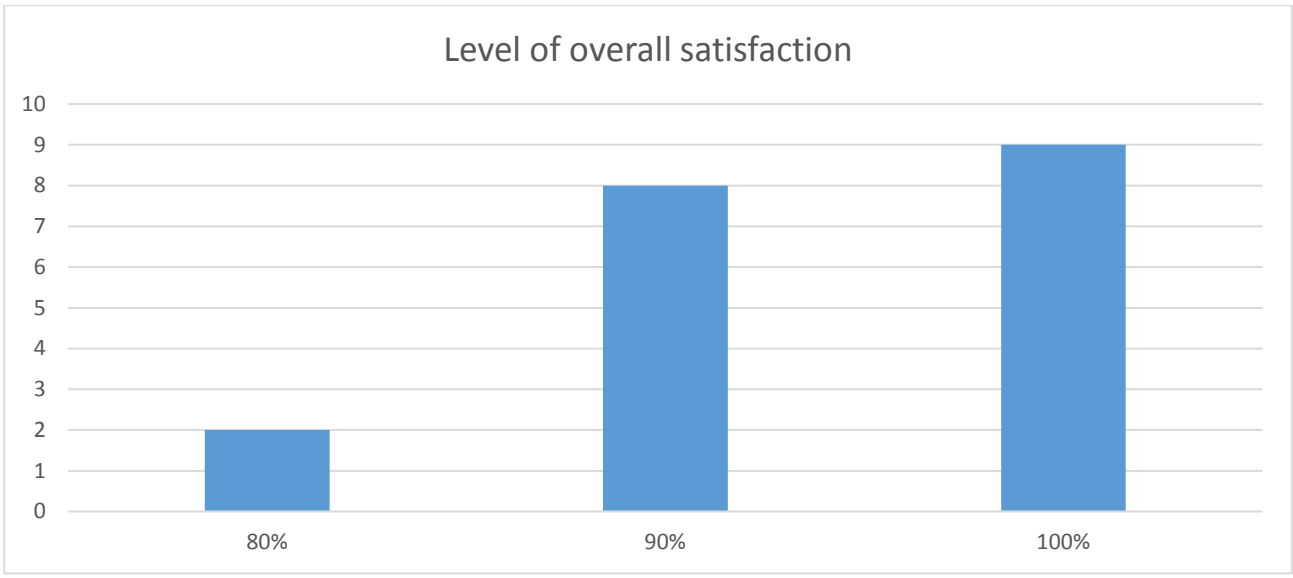
Questions and comments:

None

Workshop evaluation

A questionnaire was delivered to the participants asking to evaluate various aspect of the workshop. An overview of the 19 questionnaires retrieved is shown below. Specific comments are going to be considered for the next annual workshop organization.





Greetings and conclusions of the meeting

The next meeting will be held at the end of May 2018. It will be organized at our facilities here in Kgs. Lyngby. 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 team