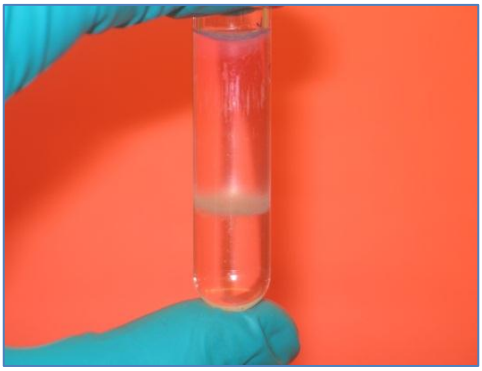
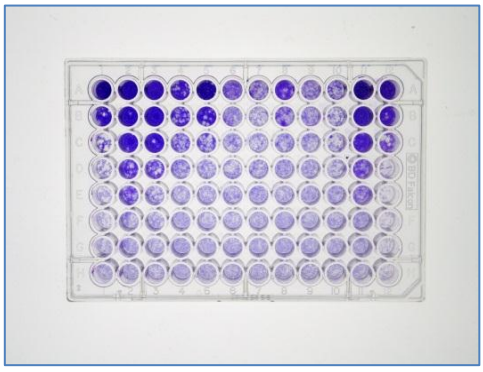
Report:
17th Annual Workshop of the National Reference Laboratories for Fish Diseases

Copenhagen, Denmark, May 29-30, 2013

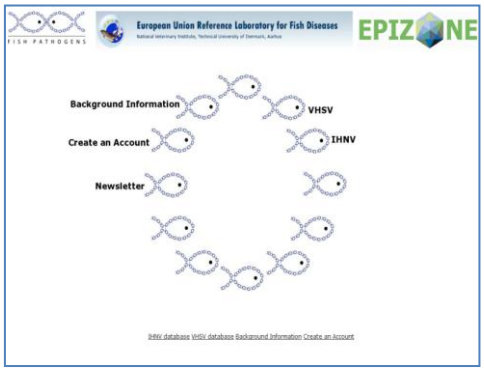
A. Salmon with petechiae in liver



Viral band on Cesium-Chloride gradient



KHV Plaque neutralisation test



www.fishpathogens.eu homepage

Organised by the European Union Reference Laboratory for Fish Diseases
National Veterinary Institute, Technical University of Denmark

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INTRODUCTION AND SHORT SUMMARY

The 17th Annual Meeting of the National Reference Laboratories for Fish Diseases was held in Copenhagen, Denmark, 29-30 May 2013 at the Auditorium of DTU Veterinary Institute in Bülowsvej 27, 1870 Frederiksberg C.
A total of 52 participants from 28 countries attended over the two days period. There were five sessions with a total of 32 presentations, 7 of which were given by invited speakers, and two round table discussions.
The scientific program of the Annual Meeting was wide and covered many different topics of current interest. The meeting was opened with the traditional session "Update on important fish diseases in Europe and their control", where participants from the Member States had the opportunity to present new findings from their home countries.
Initially an overview of the disease situation and surveillance in Europe 2012 was provided on the basis of the results obtained from the Survey & Diagnosis questionnaire.
Then the fish disease situation in Norway was presented; a detailed report in Norwegian is available at: http://www.vetinst.no/nor/Publikasjoner/Fiskehelserapporten. An English version will be available at: http://www.vetinst.no/eng/Publications/Fish-Health-Report.
The results of a survey on the impact of fish disease in the Mediterranean basin, achieved thanks to the collaboration with a panel of acknowledged fish pathologist experts, were presented.
This was followed by an update on KHV, discussing diagnostic methods for surveillance and issues related to atypical strains; after the presentation an open discussion on this disease took place with very active participation of many experts.
Then the appearance of a VHS outbreak in Scotland in a new susceptible species, the Wrasse (cleaner fish) was described.
The importance of preparation and harmonisation of legislation for fish disease surveillance and control within the EU territory was addressed with the following talk, where the Croatian Member state representative described the work in progress for the entry of Croatia in the EU.
Then the relevance of zoonoses in aquaculture was underlined by the next presentation describing *Vibrio vulnificus* outbreaks in aquaculture and their implication on human health.
This was followed by an update on non/low pathogenic HPR0 ISAV, a current issue for the salmon farming industry; the output of an EFSA specific working group on the topic was described.
Two more talks complete the first session.
First an overview on the diagnostic manuals: the tool to develop surveillance and diagnostic method for the listed fish diseases in EU was delivered.
To conclude the session the findings of a new potential emerging disease that occurred in Sweden were described.

This year the second session was dedicated to Emerging diseases. Three topics and related talks were presented.
First the Salmonid Alpha Viruses and their geographic distribution were described. These pathogens represent one of the major constraints for salmon farming in Norway.
Secondly Viral Encephalopathy and Retinopathy the main viral problem for the Mediterranean Mariculture was described.
Thirdly the outbreak of Hirame Rhabdovirus, a pathogen detected for the first time in Europe. This virus was found in connection with increased mortality in farmed grayling (*Thymallus thymallus*). The virus was identified in cooperation between laboratories as Hirame Rhabdovirus by use of molecular and immunochemical techniques.

The third session, on control and surveillance of relevant pathogens in the EU, started by a presentation on health categorization of fish farms in Europe in 2012 based on answers from the questionnaire "survey and diagnosis" that is delivered every year to all national reference laboratories.

A funded EFSA project focusing on risk ranking in aquaculture was presented and the results obtained from the questionnaire were given. The developed model calculates a quantitative risk score for individual aquaculture sites. The final calculated risk score is a value between zero and one and is intended to indicate the risk of a site relative to the risk of other sites (thereby allowing ranking). The model is suited for assessment of individual fish farms to rank farms to support surveillance to demonstrate disease freedom.

A presentation on Aquatic Animal Health Law, a piece of framework legislation, was delivered emphasizing on the issues of interest and importance for aquatic animal health.

Finally a presentation concerning EUS was given addressing how to deal with this pathogen after its delisting from the group of notifiable diseases in the EU.

In the evening a banquet dinner was held at Restaurant "*Bryggeriet Apollo*", located next to Tivoli's main entrance.

The second and last day was opened by the session on scientific research update; this fourth session faced several different topics covering molecular characterization of pathogen, a present and future core topic for all the laboratories involved in fish disease diagnosis, vaccination as a strategy to prevent disease and breeding program to select resistant fish to pathogen

The session started with an update on MOLTRAQ an EMIDA ERA-NET funded project that focus on the molecular tracing of pathogens for aquatic animals. A core goal of the MOLTRAQ project is to generate and use spatio-temporal epidemiological data, phylo-geographic data and gene expression data for important host-viral pathogen systems to identify important factors affecting the spread of diseases in aquaculture. All epidemiological and genetic data on isolates of the viruses in this project will be uploaded in the isolate database www.fishpathogens.eu. A new SAV database will also be developed and published. MOLTRAQ aims to collect and collate new data on several of the most important European aquatic pathogens and use these data to generate a better understanding of the spread of pathogens in aquatic environments.

This was followed by a presentation on sequencing and phylogenetic analysis and their application to fish pathogens, this tool is increasingly used to discriminate variants of pathogens (i.e. EHNV from other ranaviruses or HPR0 from HPRΔ in the case of ISA).

The next presentation described a infection trial with VHS in Herring (*Clupea harengus*) highlighting issues related to work in infection trial facilities with wild captured fish.

This was followed by a presentation on the results of a research collaboration with Dr. Takafumi Ito from Japan. The focus of the work was the development of a panel of monoclonal antibodies for genotyping VHSV. Unexpectedly the studies provided interesting insights in VHSV and virulence markers and mechanisms.

After coffee break, IHNV, the other rhabdovirus listed in the EU legislation was addressed, and preliminary results achieved in the MOLTRAQ project were presented.

This year another innovative topic was introduced in the program, as the interesting features of microRNAs and their implication in immune response during viral infection in fish were described, these molecules demonstrate interesting capacities modulating response to viral infection both in vivo and in vitro.

After the eradication of VHSV from Denmark in 2009 a major epidemiological study has been conducted linking disease outbreaks with epidemiological features as, geography, water source etc. The results of this study were presented.

Also the topic of vaccination, as control methods for disease in aquaculture was addressed, and two presentations on vaccination were held: Firstly, a SAV vaccination plan in Norway and secondly a project against furunculosis in marine reared rainbow trout was presented

Finally the impressing results from breeding programs selecting strains of Atlantic salmon resistant to IPNV were presented.

The annual meeting ended with the traditional update from the EURL. The results of the two proficiency tests sent out in 2012, PT1 and PT2, were presented. A report from the annual training course provided by the EURL in January/February 2013 was given and topics for next year's training course were discussed. The planned EURL activities in year 2013 were presented and proposals for the EURL work plan for 2014 were discussed.

Minutes from the meeting were taken by Drs. Helle Frank Skall, Morten Sichlau Bruun and Niccolò Vendramin, and have afterwards been sent to the presenters for correcting in order to avoid misunderstandings. The minutes are included in this report together with abstracts delivered by the presenters. Niccolò Vendramin assembled the report.

We would once again like to thank all the presenters for their great contribution without which the meeting would not have been a success.

The workshop and meeting was organised by a team consisting of Eva Haarup Sørensen, Anemone Ojala, Nicole Nicolajsen, Niccolò Vendramin 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 at the end of May 2014, more details will follow.

We wish to thank all of you for participating and look forward to seeing you next year!

Copenhagen 09 August 2013
Niels Jørgen Olesen and Niccolò Vendramin

PROGRAM

Wednesday May 29th

8:45 – 9:15	**Registration and welcome address**
9:15 – 09:30	Welcome Address and Announcements - *Niccolò Vendramin and Niels Jørgen Olesen*
SESSION I:	**Update on important fish diseases in Europe and their control**
	Chair: *Olga Haenen*, Minutes: *Helle Frank Skall*
9:30 – 9:50	Overview of the disease situation in Europe – *Niels Jørgen Olesen*
9:50 – 10:10	Update on the disease situation in Norway – *Brit Hjeltnes*
10:10 – 10:30	Update on the disease situation in the Mediterranean – *Niccolò Vendramin*
10:30 – 10:50	KHV: identification and characterization of CyHV-3– *Sven Bergmann*
10:50 – 11:10	Open discussion on KHV and its diagnosis and control
11:10 – 11:35	*Coffee break*
11:35 – 11:55	Wrasse: a new susceptible species to VHS – *Eann Munnro*
11:55 – 12:10	Implementation of EU Aquatic Animal Health Law in an EU candidate country: the case of Croatia – *Snježana Zrnčić*
12:10 – 12:25	*Vibrio vulnificus* outbreaks in Dutch eel culture: molecular genotyping, antibiotic resistance, and zoonotic impact – *Olga Haenen*
12:25 – 12:40	Output of the EFSA report on ISA – *Niels Jørgen Olesen*
12:40 – 12:55	The Diagnostic Manuals at www.eurl-fish.eu - *Niels Jørgen Olesen/Helle Frank Skall*
12:55 – 13:10	Potential emerging disease outbreak in Swedish Rainbow trout farm – *Anders Hellström*
13:10-14:00	*Lunch*

SESSION II:	**Emerging Diseases**
	Chair: *Birgit Oidtmann*, Minutes: *Niccolò Vendramin*
14:00 – 14:20	Geographic distribution of SAV in Norway – *Irene Ørpetveit*
14:20 – 14:40	Viral Encephalopathy and Retinopathy: the first viral threat for Mediterranean aquaculture – *Anna Toffan*
14:40 - 15:00	HIRRV, a new candidate for listed diseases – *Marek Matras*
15:00 – 15:35	Round table discussions on how to deal with emerging diseases – different strategies and approaches – Chair: *Birgit Oidtmann*
15:35 – 15:55	*Coffee break*
SESSION III:	**Control and surveillance of relevant pathogens in the EU**
	Chair*: Brit Hjeltnes*, Minutes*: Morten Sichlau Bruun*
15:55 - 16:15	Health categorisation of fish farms in Europe – *Niels Jørgen Olesen*
16:15 - 16:35	Risk based surveillance in aquaculture: the outcome of an EFSA project – *Birgit Oidtmann*
16:35 – 16:55	The Animal Health Law from an aquatic perspective – *Stig Mellergaard*
16:55 - 17:15	EUS and how to deal with it in the future after the delisting – *Birgit Oidtmann*
19:00 –	***BANQUET DINNER at "****Bryggeriet Apollo"* next to Tivoli's main entrance

Thursday May 30th

SESSION IV: Scientific research update

	Chair: *Richard Paley*, Minutes: *Morten Sichlau Bruun , Niccolò Vendramin*
9:00 – 9:20	MOLTRAQ – molecular epidemiology for fish diseases – *Susie Sommer Mikkelsen*
9:20 – 9:40	Phylogenetic analysis in fish diseases - *Heike Schütze*
9:40 - 10:00	VHSV in herring (*C. harengus*), does it have any importance? - *Torsten Snogdal Boutrup*
10:00 – 10:20	Molecular features of low and high pathogenic clones of VHSV Ib isolates – *Niels Jørgen Olesen*
10:20 - 10:45	*Coffee break*
10:45 – 11:05	Molecular tracing of IHNV in Europe – *Heike Schütze*
11:05 – 11:25	Fish and microRNAs – *Dennis Bela-Ong*
11:25 – 11:45	VHS epidemiology in Denmark – *Britt Bang Jensen*
11:45 – 12:05	Vaccination against SAV in Atlantic salmon in Norway - *Britt Bang Jensen*
12:05 – 12:25	Marine Vac: when applied vaccine research meet the need of the industry – *Niels Lorenzen*
12:25 – 12:45	Breeding programmes as a strategy for disease control: Atlantic salmon and IPNV – *Brit Hjeltnes*
12:45 – 13:40	*Lunch*

SESSION V: Update from the EURL

13:40 – 13:55	EURL activities in 2012 – *Niels Jørgen Olesen*
13:55 – 14:15	EURL workplan for 2013; Ideas and plans for 2014 – *Niels Jørgen Olesen*
14:05 – 14:25	EURL Training courses. Report and topics for future courses – *Susie Sommer Mikkelsen*
14:25 – 14:45	Results of the proficiency test, PT1 and PT2, 2012 – *Niccolò Vendramin*
14:45 – 15:00	Next Workshop and end of the 17th Annual Workshop – *Niels Jørgen Olesen*
15:00 – 15:15	***Coffee, cake and goodbyes***

Welcome

Niels Jørgen Olesen and Niccolò Vendramin wished everyone welcome to the 17th Annual Meeting with apologizes for the fact that some colleagues did not receive the invitation in time. 53 participants consisting of scientists from 28 countries as well as Ph.D. students from the EURL are attending the meeting. After information on technical and practical issues, the official new location of the fish disease laboratory in Copenhagen was shown and the participants were informed / warned against a music festival covering most of the town.
Niccolò Vendramin describes briefly the content of the folder distributed to all participants and on some practicalities for reimbursement.

SESSION I: Update on important fish diseases in Europe and their control

Chair: Dr. Olga Haenen
Minutes: Dr. Helle Frank Skall

OVERVIEW OF THE DISEASE SITUATION AND SURVEILLANCE IN EUROPE IN 2012

N. J. Olesen and N. Nicolajsen
National Veterinary Institute, Technical University of Denmark

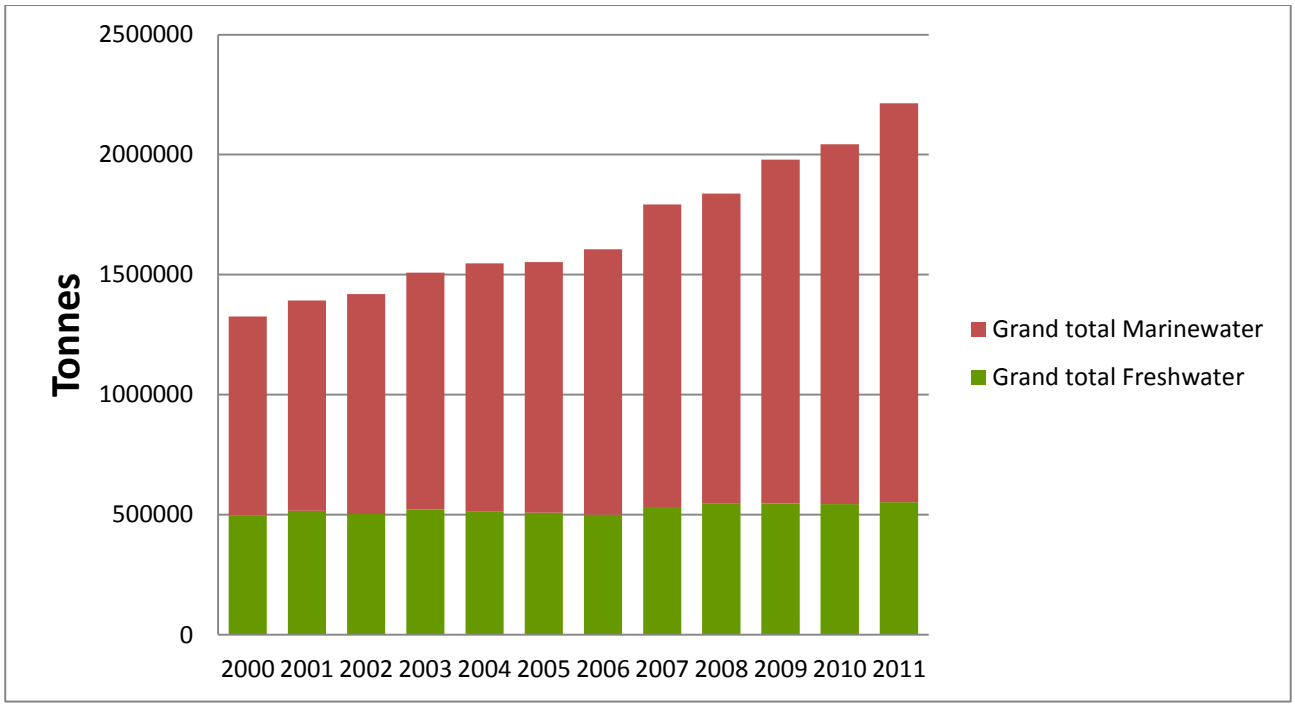
Abstract:

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. The S&D have evolved over the years, for 2012 it comprise 3 parts (not 4 parts as for 2011):

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

The data on the European aquaculture production were obtained from the FIGIS database. This database does not include information on the number and size of fish farms, and therefore these data had to be obtained directly in the questionnaire. The production has increased quite significantly from 2010 to 2011. The increase primarily account for the Atlantic salmon production, especially in Norway. With a raise from 1.6 mill in 2001 to > 2.2 mill ton in 2011, Europe is following the global development towards increased aquaculture production (Figure 1). Data from 2012 is not yet available. The farm sizes vary a lot between countries, e.g. the majority of farms in Germany produced < 5 tonnes, and for Spain the number of farms producing < 5 tonnes, 5-100 tonnes and > 100 tonnes is nearly equal.
The Atlantic salmon production has increased significantly while the rainbow trout production has stabilised in Europe in 2011. The carp production is still mainly in the Eastern part of Continental

Europe and had a slight decrease compared to the years before. Both the production of sea bream and sea bass increased in the Mediterranean countries but not at the same space as in 2002-2007.. Among other fish species of interest are pike-perch (499t), eel (6.720t), sturgeon (5.281t), cod (16.126 t), turbot (11.161t), and halibut (2.883t). Pike-perch have not yet obtained the expected increase, while the sturgeon production seems to grow significantly. The cod production decreased dramatically. Data on the health categorisation of fish farms will be given in a later presentation.
Concerning the epidemiological data, obviously, there is still a severe underreporting of VHS, IHN and KHV in many countries. Only 56 of 12.741 farms are considered VHS infected and 61 of 8.363 are considered IHN infected, while 53 of 10.403 farms are considered KHV infected in the reporting countries. There were no ISA infected farms in Europe 31.12.2012!
VHS the infection status in only known for 33% of the farms, for IHN the situation is known in 37% of the farms. While for KHV the disease situation is unknown on 95% of the farms! For farms producing Atlantic salmon and categorised for ISA, the infection status for ISA is known for 66% of the farms. The findings of ISA virus HPR0 pose some problems regarding how to health categorise salmon farms.
Many countries have surveillance programmes for SVC (19 of 35 countries), BKD (14 of 35 countries), IPN (18 of 35 countries) and *Gyrodactylus salaris* (7 of 35 countries), for which they are seeking "additional guaranties" according to §42 in CD 2006/88/EC. The number of farms in the programmes varies from very few farms to many farms. In northern European countries the most common problems are sea lice, pancreas disease, Amoebic gill disease in the salmon production, in continental Europe it is primarily bacterial diseases like ERM and *Aeromonas* infections, AGD and RTFS, while problems in the Mediterranean countries are the same as in continental except that Nodavirus infection in mariculture seem to play an increasing role.
There are very large differences between countries on how many samples are tested on cell cultures, ranging from < 100 to several thousands. The total number of samples increased since last year and PCR is coming up in most countries. The large number of PCR-tests conducted in some countries mostly reflects the KHV and ISA testing.



Total production of fish in aquaculture in Europe 2000 to 2011 (http://www.fao.org/figis)

Minutes:
For the 15th year in a row the Survey & Diagnosis investigation was conducted. In the past every country was invited to come and present their data, but as the EU has grown this will today require too much time. For this reason a summary of the data will be present to you. Concerning data of production we refer to FIGIS data, unfortunately they are one year behind, so we can only show results for 2011.
Considering most important species for aquaculture in Europe:

- Both for carp and rainbow trout the production has been quite stable.
- Seabream and seabass: there has been an increase in the production.
- For eel there has been a decrease in production over the years due to the problems of getting elvers
- The pike-perch production is still very small but seems to be rising
- The sturgeon production has been rising
- The cod production is going down
- The turbot and halibut production is going up

FIGIS does not provide any information on the number of fish farms, just the total production so for obtaining these data the contributions from the NRLs are needed.
We do not just ask you for the listed diseases but also for other diseases that may be of concern for you.
Among the countries there are big differences in the size distribution of farms. E.g. in Germany the majority of farms are small, whereas in Norway most farms are big farms. You can see all the data in the folder and they will also be uploaded on our website. These data are also interesting for epidemiologist as this is the only comprehensive data on farms and fish diseases in Europe.
There are also big differences in the fish species that are produced.
In the epidemiological data we are trying to find out how many farms that in reality are infected with the listed diseases, as the categorisation does not tell you this, as farms may de facto free even they are not categorised as free.
According to the results there were no ISA positive farms in Europe 31.12.12.
For KHV we do not know the status in 95% of the farms, whereas we know the status for ISA in almost all farms in Europe, except I norway.
The major problem in salmon production is sea lice but it seems that amoebic gill disease is emerging as well as pancreas disease in the northern countries.
IPN seems to be a decreasing problem in Norway whereas the problem is arising in Finland which used to be free.
In continental Europe amoebic gill disease also seems to be a problem.
In the Mediterranean area nodavirus is a problem.
The number of samples examined in Europe is pretty much the same in 2012 as in 2011. Germany tests a lot of samples whereas Norway do not examine many samples. The number of samples tested only by PCR is increasing.

Conclusion:
Significant increase in marine production and very stable fresh water production.
Beside this, few changes from 2011 to 2012.
Unfortunately some member states did not reply.
VHS aroused in wrasse in Scotland but ISA resolved.
VHS eradicated in Denmark.

Questions:
Eann Munro: When you are looking for the number of farms, are you looking for the licenced farms or the actively producing farms?
Niels Jørgen Olesen: We have not resolved this issue, I think the salmon producing farms should sit together and come to an agreement on how to report.
Britt-Bang Jensen: The number of samples in the graphs for Norway should be higher.
Niels Jørgen Olesen: if you find mistakes please report to us, so we can correct them, as they will be updated to the internet, so it is important that they are correct.

UPDATE ON FISH DISEASE SITUATION IN NORWAY

Hjeltnes B
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Abstract
In 2012, Norway produced 1.183.200 tons of Atlantic salmon (*Salmo salar*), 73.800 tons of rainbow trout (*Oncorhynchus mykiss*) 8.500 tons of Atlantic cod (*Gadus morhua*), 2.400 tons of Atlantic halibut (*Hippoglossus hippoglossus*) and 700 tons of other species.
Salmon louse infestation represents one of the most significant challenges to Norwegian aquaculture and increased resistance to anti sea louse chemicals is an increasing 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 louse.
The main viral problem is pancreas disease (PD), which spread to new areas in 2012. A large number of sites with disease outbreaks were reported. Reasons for this high number are the spread of marine SAV- 2 and an increase of SAV- 3 in the endemic area. However, the average mortality has decreased.
Only two cases of infectious salmon anemia (ISA) was registered in 2012. One was a brood stock facility.
Heart and skeletal muscle inflammation (HSMI) is an infectious disease in farmed salmon which has in recent years become extremely widespread. The disease was diagnosed in a total of 142 sites. The no of cases are approximately at the same level as previous years. The Norwegian Food Safety Authority has suggested HSMI to be removed from the national list.
The Norwegian Veterinary Institute diagnosed cardiomyopathy syndrome (CMS), also known as 'heart rupture' on 89 sites. This is an increase over recent years.
In 2012, IPN was diagnosed in a total of 119 sites, of which nine involved rainbow trout, with the remainder affecting salmon. The outbreaks were distributed between 30 freshwater juvenile production units and 89 marine farms. It would appear, therefore, that the prevalence of IPN in farmed salmon has fallen over the last years. New results from selective breeding and management appear to be working in relation to the IPN
Cold water vibriosis, infection with *Vibrio salmonicida,* was diagnosed on 21 marine sites from Nord-Trøndelag and northwards. The reason remains unknown, but vaccination failures, and increased infectivity load in the environment, have been suggested as possible explanations.
Amoebic gill disease, *Neoparamoeba perurans* (AGD) was diagnosed in five sites in the South-West part of the country, but with very low mortality. Screening revealed that several sites were infected. The amoeba has previously been reported in Norway in 2006.
Production losses remain a significant problem in Norwegian aquaculture.

More information can be found at:
http://www.vetinst.no/nor/content/download/10605/134088/file/2012_Fiskehelserapporten_web.pdf

Minutes:
Survey & Diagnosis is a very good tool and it is extremely useful.
Norway is the main producer of Atlantic salmon in Europe that is the most important species by production and value and therefore most of the presentation will be on salmon although other fish species are currently produced in Norway.

ISA number of outbreaks has gone down from 10 outbreaks in 2009 to 2 in 2012, but unfortunately we have had a couple of suspicions in the last weeks. The ISA situation seems to have been solved in the problematic northern area. The OIE has decided to differentiate between HPR0 and the HPR-deleted isolates. Both will be listed but the HRP0 will probably not impose trade restrictions.
SAV has been increasing over the years in Norway with a reduction in the later years but again going up, with a local epidemic situation in an area of Norway.
HSMI is wide spread and is still included on the national list but may be excluded. It is coupled to a reovirus (SRV) which is detected also in healthy fish, both wild and farmed.
CMS affects large market-size fish. The accumulated mortality is seldom above 20% and is associated with a new totivirus: piscine myocarditis virus (PMCV). PMCV is only detected in fish suffering from CMS.
IPNV is evenly distributed over the country but the number of outbreaks has gone down. This is probably caused by both increased resistance breeding and better management techniques.
Vibrio salmonicida was causing a lot of antibiotic use which was resolved when vaccination was introduced in 1987. But in 2012 and 2013 there has been a significant increase in the number of farms (app. 20) infected with *Vibrio salmonicida*. It may be caused by vaccination failure as the pharmaceutical companies has lowered the antigenic compound as this produces some side effects, but this does not really seem to be the problem.
Flavobacterium infection may go into the national list.
Salmon lice have been treated with chemical compounds since 1978 with new compounds in 1990 and 2000 with increased resistance from 2007. Today it is a big problem.
Neoparamoeba perurans has now been detected in Norway causing amoebic gill disease. There are infected sites in the southern part of Norway.
The production losses have gone down from app 35% in the 80-ties and today are around 20% but I think this number is too high. Scotland and also individual farmers in Norway has shown that this number can be reduced to 10%.
Conclusions:
ISA is controlled.
PD has increased in reported cases and has spread to new areas. The average mortality is reduced.
HSMI remains stable.
CMS is increasing.
You can find more information in the fish health report on the internet. The English version will hopefully be available within a month.

Questions:
Niccolò Vendramin: Concerning the cleaner fish, do you have a surveillance program for these?
Brit Hjeltnes: we do not have a specific surveillance program but we have a lot of programmes with the farmers.
Henrik Korsholm: Have you seen CMS in other species than salmon
Brit Hjeltnes: No, to my knowledge only in salmon.

UPDATE ON FISH DISEASE SITUATION IN THE MEDITERRANEAN BASIN

Vendramin N.[1]

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Abstract:
The Mediterranean basin represents an interesting area for aquaculture. Beside ?? historically established salmonid (rainbow trout, brook trout and charr) and carp farming, mariculture (sea cages aquaculture) has developed fast in the last 20 years and the production is estimated to be around 1,5 Million Tonnes per year (FIGIS 2011).
The aim of this study was to start and establish a platform to share information and to communicate with authorities and stakeholders in order 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 the marine and fresh water sector was delivered to a panel of 20 experts.
13 questionnaires were delivered for Seawater Aquaculture, while 10 questionnaires were filled for freshwater environment. Data are here presented according to the ranking.
Marine Environment:
- Viral disease: 8 out of 13 experts listed VER as the most important disease in the Mediterranean, with some specific request for development of a commercial vaccine and certification of diagnostics methods through a specific ringtest.

- Bacterial diseases: the disease characterized by highest impact is Tenacibaculosis, old known vibriosis and pasteurellosis remain main characters in Med. Mariculture.

- Parasitic disease: emerging high impact caused by enteromyxosis, isopods and monogeneans (*Cryptocarion irritans and Amyloodinium ocellatum*) and gill flukes (*Diplectanum aequans and Sparicotyle chrysophrii*) mainly present in inland farms (earth ponds and concrete tanks based).

- Unknown aetiology: Winter Syndrome, a dismetabolic disease, is considered to produce huge impact in Sea bream fish farming not because of mortality, normally ranging from 5 to 15% in 1 year old fish but for the growth reduction. Petechial rash syndrome appears in sea bream.

Fresh water environment:
- Bacterial disease: Rainbow trout fry syndrome (RTFS) responsible for significant mortalities in rainbow trout (*O. mykiss*). during juvenile stages, particularly if not treated promptly, RTFS become a limiting constraint for trout farming development requiring the need of an efficacious vaccine. Furunculosis causing high damage both to rainbow trout and other salmonids (salmo trutta, salvelinus fontinalis, salvelinus alpinus). Lactococcosis in rainbow trout reared with warm water temperature (>16°C) in market size.
- Viral disease: including fish rhabdoviruses (IHN and VHS) but also IPN and Salmonid Alphavirus.

- Emerging unknown aetiology disease: RTGE and Red Mark Syndrome are becoming more and more important.

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Minutes:
As the S&D could be affected by different bias I have tried to make a more general overview of the situation in the Mediterranean. The Mediterranean Basin is connected not only to Europe but also to Africa and Asia.
Approx. 1,5 million tonnes of finfish production every year in the Mediterranean area, and as the weight of the fish produced is typically 350 g, this is really a lot of fish, compared to salmon production where the weight of harvested fish is e.g. 5 kg.
The marine fish production is the most important in the Mediterranean area.
A number of known experts in this field consisting of private vet consultants as well as official laboratories have been contacted and a questionnaire was sent to them. In the questionnaire the experts were asked which diseases were the one characterized by the highest economic impact. 20 experts were contacted and 13 questionnaires were received from all the countries covering the Mediterranean basis except Africa.
Viral Encephalopathy and Retinopathy (VER) is so far the most important diseases in the marine Mediterranean aquaculture. Sea bass remain the target species mainly at larval/nursery stage, with implication for market size as well. Others species are also infected. There is a need for a vaccine and a recognised certification for PCR test performed to check fry batches.
No matter that there are treatments and some vaccines bacterial infections are still a problem: *Vibrio, Photobacterium damselae* subsp *piscicida*) and zoonotic bacteria.
Parasites are also a problem. The isopods migrate into the mouth of the fish and makes it impossible for the fish to eat.
An emerging disease is petechial rash disease.
In the freshwater sector, VHS is not being considered a great problem, this data may be biased because of a large number of farms in category 3. But for the other diseases the results are trustworthy. Lactococcus is a problem and also the rainbow trout gastroenteritis (RTGE) which is a problem in market size fish.
I will try to keep this work going and if someone knows of experts that should be contacted please tell me.

Questions:
Snjezana Zrnčić: with the listed diseases we know where to send samples, but where can we send samples for the non-listed diseases?
Niccolò Vendramin: We hope we in the future will be able to make a PT3 covering non-listed diseases.

THE KOI HERPESVIRUS (KHV): IDENTIFICATION AND CHARACTERIZATION OF Cyprinid Herpesvirus 3 (CyHV-3)

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Abstract:
Over the last years, more and more severe outbreaks of koi herpesvirus (KHV) disease (KHVD) in common carp or koi (*Cyprinus carpio*) but also detections in latently infected fish without clinical signs are caused by viruses which cannot be identified by diagnostic polymerase chain reactions (PCR) recommended by the OIE. In the EU a draft based on an "EPIZONE workshop" for diagnostic PCR for KHV detection was written and recently discussed
The OIE recommends a PCR according to Bercovier et al. 2005 followed by a nested PCR (CEFAS, Dr. Keith Way) and a PCR according to Yuasa et al. 2005, which represents an improved PCR according to Gray at al. 2002. Due to the relatively low analytical sensitivity requiring a concentration of at least 102 to 104 particles per ml, 20 to 30 % of KHVD outbreaks in Germany are not detectable by these PCRs. Often, false-negative test results occur. To increase the analytical sensitivity, especially with respect to samples from latently KHV infected fish, the sampling procedure must be adapted, if the EU still wants to pursue the goal of KHVD eradication.
One possibility could be the establishment of different PCRs and / or real-time PCRs with an analytical sensitivity of 5 to 10 particles per ml. This is the concentration that is found in healthy but latently KHV infected fish. The additional use of serological assays such as antibody ELISA, immunofluorescence assays, or serum neutralization assay using fish sera could be very helpful. These assays were validated in the EU project "EPIZONE WPs 6.1 and 4.5".
Recently, we designed new primer pairs over spanning the region of interest where the recommended PCRs, e.g. PCR according to Gilad et al. (2002, 2004), Bergmann et al. (2006) and / or Bercovier et al. (2005) including the unpublished nested PCR, produced negative results with samples from an acute outbreak with typical clinical signs in carp or koi. These results were compared after sequence analysis of fragments obtained from a PCR (CEFAS, Dr. D. Stone) recognizing most of the Alloherpesviridae using the viral DNA polymerase gene (pol). It was shown that the sequence obtained from pol differs by up to five per cent from the published sequences. PCRs which recognize other KHV genes or have a higher analytical sensitivity, e.g. phosphate membrane gene PCR (Bergmann et al. 2009), semi-nested PCR (Bergmann et al. 2010), ORF 81 or 99 genes (unpublished, Dr. H. Schütze), generated positive signals with these samples.
It seems to be very important that different PCRs recognizing different genes are included into KHV diagnostics. Serological assays were used for pre-screening of farms.
Last but not least, sample collection (lethal but also non-lethal) is the most important step in KHV diagnostics. KHV concentration (1 – 10 particles per ml in latently infected fish) can be increased by separating the fish used for sampling for a duration of 24 h to 4 days before collection.

Minutes:
This disease generates a number of problems for diagnostics, these problem starts with the sampling procedures. The OIE recommends samples directly collected with up to two fish per pool in DNA-zol, our procedure suggest that we pool up to five fish and use silica-matrix based purification.
For Surveillance the OIE recommend five fish pools whereas in Germany we recommend 2 fish pools and separation of fish 24 hours to 4 days before sampling.
For diagnosis OIE recommend gross and microscopic pathology, smears from kidney, liver, brain (IFAT), cell culture isolation, PRC according to protocols provided by Bercovier and Yuasa ,

serology, while no nested PCR is recommended. On the contrary we recommend nested PCR, pathology, cell culture is not worth doing. The Yuasa PCR is not very sensitive.

Concerning the OIE recommendation:

- when there is high virus load the OIE recommend up to two fish in pool
- on the contrary when there is low virus load OIE recommend up to five fish in a pool.

This might generate false-negative results due to the pooling system since the diagnostic sensitivity of PCRs is not always sensitive enough.

Considering on the other hand the EU legislation the following issues should be addressed in the next future:

- As a rule no sample collection is necessary
- Only inspection of farms by vet authorities once a year
- No active search for agents in a surveillance programs
- Samples are taken freshly from fish, no real advise for latency
- Only *Cyprinus carpio* is the target

With these recommendations no eradication of KHVD is possible!

Additional problems over the last years:

Atypical KHV inducing KHVD tested negative with recommended PCRs , on the other hand they provide positive results with generic primers. Differences up to 5% to published sequences for KHV.

Should atypical KHV inducing KHVD symptoms with severe losses in *Cyprinus carpio* be called KHV?

What about KHV in roach?

Conclusions:

In cases of serious KHVD there are no problems in detecting the typical viral strain.
In cases of surveillance and latency it is very difficult to exclude KHV
The sampling procedures should be standardised
The sensitivity of the recommended diagnostic assays is not suitable for the implementation of effective surveillance program. A definition of KHVD is urgently needed
Molecular diagnostics should be combined with serology

Ongoing work:

Sequence analysis for fragments with overlapping primers for samples which are negative by PCRs according to Bercovier, Yuasa and Bergmann.
Experiments to isolate atypical KHV.
Proper validation of serological assays
It is necessary to harmonize the sampling procedures and to establish a real KHV network.

Questions:

Olga Haenen: We get samples directly from Schipol Airport, and we also find atypical KHV but with no signs in the fish.
Richard Paley: We also find atypical KHV in England but also with no signs of disease.
Sven Bergmann: In our cases we do have symptoms, gill necrosis, patches on the skin, enophthalmus but we do not find the typical KHV, only atypical KHV.

Brit Hjeltnes: So what you say is that the atypical host range is broader?
Sven Bergmann: No, the host range is broad both from the typical and atypical. We can identify these viruses from at least 7 species.
Richard Paley: Are you able to identify by other means than PCR.
Sven Bergmann: yes, by in situ hybridisation.
Niels Jørgen Olesen: The questions somehow get more and more blurred as we learn more.
Sven Bergmann: In roach we cannot be 100 % sure that the atypical KHV caused the disease but we can find much virus in the roach.
Olga Haenen: Concerning the sample size, it would be too costly if you only can pool two fish.
Sven Bergmann: But the problem is the latency, if they are not stressed there is a 90% chance to find the fish negative and then if you also pool the fish... if the fish are stressed there is a 90% chance to find the fish positive.
Birgit Oidtmann: We know a lot about the analytical sensitivity, but we do not know much of the diagnostic sensitivity when we look at the whole chain from taking the sample and up. Which kind of sampling should we partake, that we do not know. We need projects looking into this, so we can feel confident in what we are doing.
Sven Bergmann: How do we handle changing pathogens? I talked with a colleague working with mammals and the told they are developing new PCRs every week to keep up with the virus evolving.
Olga Haenen: What I hear is that there is a need for a KHV group in Europe and an EU project.
Brit Hjeltnes: What will be your recommendations to the OIE? What I heard you saying is that we cannot trust the methods. For surveillance we do not have the tools?
Sven Bergmann: We do have the tools but we are not allowed to use them. We should use serology which works nicely. We can't do eradication without serology. So first serology and then if positive signal we should go and look for the virus.
Richard Paley: Serology does work but is not very used in fish diseases.
Sven Bergmann: You get titres rising 1000 times within a fortnight.
Birgit Oidtmann: At the moment the OIE reference labs are the one that should make recommendations for surveillance. One reason that the serology is not included may be due to the fact that probably many laboratories will not be able to do serology at the moment. Furthermore the diagnostic manual is sent out for review to many countries and changes may be implemented accordingly, and in fact it used to be 2-fish-samples for surveillance but a country wanted this changed due to costs.
Stig Mellergaard: Are the diagnostic tools sufficiently developed to support an eradication programme? And is it really realistic to eradicate a herpesvirus from an area when it has been introduced?
Sven Bergmann: We do have the tool for eradication: serology, but we can't use it at the moment. For eradication you cannot compare mollusc with fish, a fish we can take out of the water but we cannot do this with the molluscs – this is a difference to oyster herpesvirus.
Niels Jørgen Olesen: Some years ago a nice report was made on recommendations for KHV but we have learned more since then and we need to look at the recommendations again. For serology we need to validate the methods according to the OIE recommendations which are a huge work.
Sven Bergmann: We have validated the ELISA according to OIE recommendations and you will be able to read about this soon.

AN OUTBREAK OF VIRAL HAEMORRHAGIC SEPTICAEMIA (VHS) IN WRASSE COHABITING WITH ATLANTIC SALMON IN THE SHETLAND ISLES, SCOTLAND

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Abstract:
Viral haemorrhagic septicaemia (VHS) is an infectious disease of farmed and wild fish and has an extensive host range in both freshwater and marine environments. The causative virus (VHSV) is an enveloped negative-strand RNA virus belonging to the genus Novirhabdovirus, within the Rhabdoviridae family.
In December 2012, a wrasse population consisting of Ballan (*Labrus bergylta*), Corkwing (*Crenilabrus melops*), Cuckoo (*Labrus mixus*), Goldsinny (*Ctenolabrus rupestris*) and Rockcock (*Centrolabrus exoletus*), held at a seawater hatchery in the Shetland Isles, experienced a mortality event. Approximately 10,000 wrasse were being held at the facility on behalf of an Atlantic salmon (*Salmo salar L.*) aquaculture company for use as a means of biological control as part of their sea lice management strategy.
Diagnostic samples were sent to a third party and reported as VHSV positive by real-time reverse transcriptase PCR (qRT-PCR). Fish Health Inspectors from Marine Scotland Science were immediately informed and movement restrictions were applied on the suspect site, the site that supplied the wrasse and 16 A. salmon sites stocked with wrasse that were cohorts to those at the suspect site. Statutory sampling of the aforementioned sites was conducted as required by the Aquatic Animal Health (Scotland) Regulations 2009, in accordance with EU Council Directive 2006/88/EC; 30 fish test from the suspect site, 150 fish sample taken from all susceptible species stocked on the supplying site and 15 wrasse per site from the 16 sites containing cohorts to the suspect site. The 16 sites were initially considered as one population with an increased sampling regime being implemented if VHSV were detected.
The site of initial suspicion was confirmed VHSV positive by virus isolation followed by ELISA (30/30) and by qRT-PCR (27/30). The supplying site screened negative and all Scottish mainland and Western Isles sites (11/16 sites) stocked with wrasse cohorts also tested negative. However, the additional 5 sites, all located within the Shetland Isles, screened VHSV positive. Further testing of the 11 sites on the Scottish mainland and Western Isles at the 150 wrasse level produced negative results while 150 cohabiting A. salmon from each of the 5 positive sites in Shetland were screened for VHSV and produced negative results.
Nucleic acid sequencing of the N- and G-genes was conducted and all isolates were > 99-99.8% similar at the nucleotide level and phylogenetic analysis determined that they belong to Genotype III. This suggests that the infection is uniquely connected to Shetland.

Minutes:
In December 2012 there was a suspicion of VHS at a seawater hatchery site in Shetland. Mortality occurred in a fish population consisting of 10000 wrasse specimen. Samples were sent to a third party and reported as VHSV positive by qRT-PCR. Restrictions were placed on the hatchery site and the supplying site and on sites with cohorts.
The site of initial suspicion confirmed VHSV 27/30 qRT-PCR, 30/30 from virus isolation of BF-2 and FHM.

It is important to underline that Wrasse is not listed as a susceptible species.

Output of the analysis carried out on different sites:

1) Analysis from the Supplying site: wrasse originated from wild caught populations, some cultured stocks. 150 fish statutory sample susceptible species. The wild caught were collected by one fisherman using one boat around the same time in the neighbourhood. There were no clinical signs of disease.

2) 16 Seawater cage sites also had wrasse populations (cohorts to the suspect site) from the supplier. 15 fish per site were tested. All sited on the Scottish mainland and western isles screened VHSV negative. Wrasse on 5 commercial marine Atlantic salmon farms in the Shetland Isles tested VHSV positive. Cohabiting *A. Salmon* stocks from these 5 sites tested VHSV negative (150 fish/site), 60 mixed gadoids cohabiting with salmon from 1 of the VHSV infected farms were sampled, one poor cod was found positive. It is foreseen to test 60 mixed gadoids every quarter of year from the farms in the future.

3) Wild fish survey – Shetland

Wild marine species were samples following demersal trawling (april 2013). Fish samples in proportion to the number of species caught and screened forVHSV in pools of 10 by virus isolation. 11/140 were VHSV positive. VHSV was found in gurnard (1/3), herring (4/22), and plaice (1/40)

The wild wrasse were caught by a single fisherman from a single area and transported either to a holding place or directly to the farms. The fish from the holding place were then transported by 3 transporters to the Shetland Islands and it was from these fish the positive samples were found.

Using the distances modelled for transmission of VHS due to the infected wrasse on marine cage sites, a protection zone of 0.5 Km may be appropriate. The surveillance zone could be set at double this distance i.e. 1 Km, which could be considered consistent with the principles of the advice provided in Commission Decision 2003/266/EC and the draft diagnostic manual for Council Directive 2006/88/EC.

All isolates group within VHSV genotype III. Wrasse isolates >99% similar on nucleotide level. 10/11 wild fish isolates were identical. Comparable variation between wild fish isolates and wrasse isolates where the same as within wrasse isolates.

Wrasse at the marine hatchery were culled; the removal programme commenced the day after at the 5 remaining sites. 2 sites are now depopulated, farm records and statistical analysis indicates 99%
Lumpsuckers were held as part of sea lice control on VHSV positive site. There were heavy mortality with up 6% per day, *Listonella anguillarum* was isolated from the fish, no VHSV was isolated. Antibiotics helped.

Origin of the outbreak?
It is most likely that the outbreak originated from wild fish around Shetland.

Questions:

Stig Mellergaard: Do you think it will be possible to use wrasse in the area in the future with the wild fish VHSV being so high?

Eann Munro: we know there are herring spawning ground in the area so it will be a question if we can use them.

Sven Bergmann: how soon did the samples become positive on cell culture?

Eann Munro: most of them were positive in the primary culture.

IMPLEMENTATION OF EU AQUATIC ANIMAL HEALTH LAW IN EU CANDIDATE COUNTRY: THE CASE OF CROATIA

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Abstract:
Croatia is with its southern part Mediterranean country while with its northern part it belongs to the Central Europe. Aquaculture sector is diverse and depends on natural conditions. Warm water freshwater aquaculture is situated in the continental (northern) part of the country and the major produced species are common carp (*Cyprinus carpio*), other cyprinid species, catfish (*Silurus glanis*), pike (*Esox lucius*) and pike perch (*Zander lucioperca*). Salmonid farms are in the mountain region cultivating rainbow trout (*Onchorhynchus mykiss*) and brown trout (*Salmo trutta* m. *fario*). Along the 5800 km of Adriatic coastline there are about 60 farms of sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) which represents the most valuable and the largest production in the country. In the recent decade some tuna (*Thunnus thynnus*) farms have been established and this production is based on the fattening captured fish of different size, age and origin according to ICCAT's rules and depends mainly on global market. Cultivation of molluscs' species; oysters (*Ostrea edulis*) and mussels (*Mytilus galloprovincialis*) has the longest tradition but the production is for the moment low and sufficient for the demands of the local market. Overall annual production in fresh water and in the sea is about 18.000 tons and the consumption of fish is rather low, about 10 kg *per capita.*
Control of listed diseases is performed according to the "Decree on the measures of animal health protection against infectious and parasite diseases" issued yearly by Ministry of Agriculture and based on the Council Directive 2006/88/EC which has been adopted in 2008 in national legislation. Diseases surveillance programme comprise control of VHS/IHN on salmonid farms, KHV on cyprind farms, bonamiosis and marteioliosis on oyster farms and marteiliosis on mussels farms. There are several ongoing programmes like risk assessment, authorisation of APBs, antimicrobial resistance baseline studies on the most prevalent bacteria in salmonids and marine fish and their resistance which have been initiated this year.

Minutes:
Croatia is a small country. Concerning aquaculture production and related hydrographic basins there are 3 different parts: the Danubian watershed, the Adriatic watershed and Adriatic sea Basin.
Seabream and seabass is the biggest production. Also cyprinid and salmonids farms are relevant, moreover we have a small production of oysters. Salmonid farms are mainly situatited at the mouth of the rivers. Mariculture is the most important production. Also meagre and sharpsnout sea breams and dentex are produced besided sea bass and bream.
Mollusc farming is the oldest farming done: mussels and oysters. Tuna cultivation is decreasing lately, it is just fattening that is done, the small tuna is caught in the wild. They are feed by local herring and sardines and by imported fish.
The mollusc farms are many 246 but small, tuna farms 7 farms, seabream/bass 57, trout 30 farms.
The legal framework has been implemented based on the CD. We have started with authorisation of APBs according to the directive. Recording obligation: traceability, good hygiene practice, animal

health surveillance. The competent authorities are educated to perform risk assessments using a common form.
Targeted surveillance programmes have been done in 2010, 2011 and 2012. In total 30 farms have been authorised and approximately 20 have been regularly sampled. IPNV identified, no VHSV, no IHNV, no KHV.
Concerning mollusc farms, Marteiliosis has been identified but not bonamiosis.
A program for surveillance of antimicrobial resistance has been set up now.

- Baseline study on prevalence of *Yersinia ruckeri* and *Flavobacterium psychrophila* and their resistance to more often used antimicrobials in salmonids
- Baseline study on prevalence of *Listonella anguillarum* and *Tenacibaculum maritimum* and their resistance to more often used antimicrobials in marine cultivated fish (sea bass and sea bream)

Questions:
Niccolo Vendramin: How is the plan for antibiotic resistance structured?
Snjezana Zrnčić the project has been funded by the ministry, bacteriological samples will be collected on the farms and antibiotic resistance will be evaluated.

VIBRIO VULNIFICUS OUTBREAKS IN DUTCH EEL CULTURE: MOLECULAR GENOTYPING, ANTIBIOTIC RESISTANCE, AND ZOONOTIC IMPACT

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Abstract:
Vibrio vulnificus is an aggressive potentially zoonotic bacterial pathogen of fish. In this study, from 1996-2009, *V. vulnificus* was isolated 24 times in the Netherlands, as causative agent of serious eel disease outbreaks (23 strains) with high mortalities at 9 indoor eel farms, of which one outbreak was related to a severe zoonosis of the eel farmer (necrotic fasciitis, 1 strain).
In this study, we tested the genetic relatedness of the 24 mentioned strains of *V. vulnificus* by two genotyping techniques, MLST (using HiMLST technology) and rep-PCR (DiversiLab®). Additionally, the antibiotic resistance was tested.
The 24 strains could be separated into 8 HiMLST types and 8 rep-PCR types, which corresponded almost exactly to each other. Both methods were appropriate to distinguish a zoonotic strain form the other eel pathogenic strains of this study. Only one of the 8 HiMLST types was present in the online MLST database and the other eight each had one or more new allele variations (ST 137-143). This indicates that many, yet unknown *V. vulnificus* genotypes occur in eel farms. Most farms harboured a single genotype, and most genotypes were restricted to a single farm. However, two farms harboured two genotypes in time. The eel farmer that suffered a zoonosis from *V. vulnificus* carried the same genotype as his diseased eels, a demonstration of the zoonotic potential of this strain (HiMLST ST 112).
The antimicrobial resistance patterns were diverse amongst the genotypes of *V. vulnificus* and no clear correlation was found between genotype and antibiotic resistance profile of a strain. Most *V. vulnificus* strains were resistant to cefoxitin and showed multi-resistance to quinolones, properties that seemed to develop after prolonged use of flumequin bath and other antibiotics in the eel farms.
Although V.vulnificus related contact zoonosis from fish are scarce, individual cases may be severe, especially in immuno compromized patients. Risk assessment and prevention are needed to protect fish farmers and fish processors against V.vulnificus infections, particularly from eels. Medics should be aware about the potential zoonotic risk of V.vulnificus infections in our geographical area, associated with indoor eel farming.

Minutes:
More than 30 farms rear eels in the Netherlands the production is around a few hundreds of tons per year. The stocking is always with wild elvers. The water temperature is high and people work with bare hands.
There are three different biotypes of *Vibrio vulnificus*, in eel biotype 2, tilapia type 3, many marine fish mostly benthic mostly type 2, sediments type 1.
In September 2007 a farmer was infected (with strain classified as ST 112) after having an outbreak in august in his farms. He had not used DPI when removing the fish .
The year after the farm still had *V. vulnificus* ; in this case the strain ST 140 have been identified and a different pathological picture appeared.

The farmer was infected is his hand very severely and went to surgery and treated with antibiotics; the farm went bankrupt but the farmer survived.
The zoonotic strain has been present since 2005 in the farm as shown by hiMLST, Diversilab (R).
Antibiotic resistance testing was done on the isolates. The resistance pattern varied, most resistant against cefoxitin. The zoonosis strain showed resistance against quinolones.
ST 112 seemed to spread and act from inside the eel, whereas ST 140 more from the outside inwards.
The impact of *V. vulnificus* is big both to ells and man.
A scientific paper is in preparation for the Journal Diseases of Aquatic Organisms.

Questions:
Anders Hellström: we have had infections in ell farms but not in the farmers. But in warm summers we have seen it in swimmers.
Inger Dalsgaard: we have had cases in fishermen, and we have also had a case in a fish farmer.
Olga Haenen: we have not had cases in swimmers but we have had cases from food**.**

SCIENTIFIC OPINION ON INFECTIOUS SALMON ANAEMIA (ISA) EFSA PANEL ON ANIMAL HEALTH AND WELFARE (AHAW)

European Food Safety Authority (EFSA), Parma, Italy

Presented by N. J. Olesen
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Abstract:
Atlantic salmon is the only species in which the disease infectious salmon anaemia (ISA) has been observed naturally. Initial reports of findings of infectious salmon anaemia virus (ISAV) before 2002, did not distinguish between non virulent HPR0 and virulent HPRΔ viruses, thus making interpretation of older findings difficult in the light of current knowledge. Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the relationship between HPR0 and HPRΔ, the risk of HPRΔ ISAV emerging from HPR0 ISAV, and possible risk factors for such an emergence. HPR0 ISAV does not cause clinical disease in Atlantic salmon; however, it causes a transient subclinical infection and replicates mainly in gills. There is no evidence for HPR0 ISAV leading to natural infection and replication in fish species other than Atlantic salmon. Virulent ISAV have deletions in the HPR region of the HE gene and they have either an insertion or the Q266L mutation in the F gene. The most plausible hypothesis is that virulent ISAV (HPRΔ) is derived from HPR0 ISAV. This is further supported by the close association between the genetic relatedness and spatio-temporal distances of virus strains in solitary outbreaks. Epidemiological and historical data from solitary disease outbreaks indicates that the risk of HPRΔ ISAV emerging from HPR0 is low, but not negligible. The risk factors for HPRΔ emergence from HPR0 are unknown. Nevertheless, any factor that affects virus replication or host susceptibility could possibly influence the risk of emergence. More research is needed on the drivers for transition from HPR0 to HPRΔ and factors affecting host susceptibility and thereby emergence of clinical disease. A quantitative assessment of the different evolutionary forces for ISA would be useful, as well as the prevalence of ISAV HPR0 in farmed and wild Atlantic salmon.

The entire text of the Scientific opinion is available online at:
http://www.efsa.europa.eu/it/efsajournal/doc/2971.pdf

The scientific members of the Working Group on ISA were: Edgar Brun, Debes Christiansen, Niels Jørgen Olesen, Rob Raynard, and Espen Rimstad.

Minutes:
It was requested to look into the HPR0 topic and how should we deal with it within the legislative framework, how shall we deal with the HPR0 and HPR-deletions in the future. There is the need to know which risk factors prompt the emergence of pathogenic isolates. We know that HPR0 do give a transient infection in the gills without clinical signs. The HPR0 can be differentiated from the HPR-deletions by sequencing. No clinical disease has been seen with HPR0 and HPR0 is considered as the "natural" or background isolate. The only species where HPR0 has been found is Atlantic salmon and it has been found in both wild and farmed salmon. The pathogenic isolates have deletions in segment 6 and changes in the fusion protein. The emergence of pathogenic isolates from HPR0 is low but not negligible. So having HPR0 is a risk. It is unknown what triggers the deletions but it is known that good biosecurity measures will lower the risk. It is unknown if infection with HPR0 will provide some kind of immunity against HPR-deleted viruses.

The working group came out with the conclusion that risk of emergence of HPR-deleted depends on replication of HPR0 in the susceptible host. Any factor that affects replication or host susceptibility would, therefore, also influence the risk of emergence of HPR-deleted ISAV.
The situation is similar to avian influenza and we should look into how they deal with it.

Questions:
Olga Haenen: So HPR0 should be reportable.
Niels Jørgen Olesen: If you can show you do not have HPR0 you should be able to claim additional guaranties.
Brit Hjeltnes: you cannot claim freedom for HPR0 based on historical background. You would have to design a specific program for this and you would have to consider if it is worth doing it.
Stig Mellergaard: Could we deal with isolates that would switch?
Niels Jørgen Olesen: Many different ISA outbreaks seem to originate from different HPR0 viruses and there is a risk. But usually no direct link between ISA-deletion and HPR0 in the environment can be made..
Heike Schütze: How is the status in France for ISA? We have been asked to test samples for ISA from France, but is this necessary?
Stig Mellergaard: All European countries are free from ISA, so legislative wise there is no need to test samples from France to verify that they are free.

SURVEILLANCE AND DIAGNOSTIC METHOD FOR THE LISTED FISH DISEASES IN EU

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Abstract:
A Commission Decision has been in preparation for several years in order to implement Directive 2006/88/EC as regards requirements for surveillance and diagnostic methods, with the objectives of implementing:

1. preventive measures relating to the surveillance and early detection of the fish diseases listed in Annex IV
2. Requirements to achieve the status of disease free Member State, zone or compartment
3. Diagnostic procedures, sampling methods and criteria for the evaluation of the results of laboratory tests that may lead to a suspicion, confirmation or to achieve the status of disease free zone or compartment.

Recently the 4th draft of the Decision appeared, the draft is divided into:

PART I: Introduction and objectives

PART II: SURVEILLANCE AND DIAGNOSTIC METHODS FOR VHS AND IHN
Aetiology of VHS and IHN
Provisions for programmes to achieve and to maintain certain health statuses with regard to VHS and/or IHN and to contain VHSV and/or IHNV infections

I. Diagnostic methods
III.1. Organs to be sampled
III.2. Diagnostic methods to achieve and to maintain disease-free status of VHS and/or IHN
III.3. Sampling and diagnostic methods to rule out and to confirm VHS and/or IHN

The detailed protocols for the diagnostic methods and the evaluation of their results as applied by the EURL can be found in the following web-site: www.eurl-fish.eu

PART III: SURVEILLANCE AND DIAGNOSTIC METHODS FOR KHV

PART IV: SURVEILLANCE AND DIAGNOSTIC METHODS FOR ISA

The draft still need to be assessed more in details and to be finally accepted and implemented

Minutes:
A new Commission Decision is in preparation. The reason is to merge the old Commission Decision stating how to sample and diagnose listed diseases with 2006/88. Sigrid Cabot left last autumn and was substituted by another person that now has been substituted again. This has probably slowed down the process.
The new parts will consider:

1) the aetiology of the diseases in question
2) the provisions for programmes to achieve and to maintain disease freedom.

For now we have a problem regarding KHV as what we have today is more or less a direct translation of what we have for VHS/IHN into KHVD.
The diagnostic measures will be those that are given by the EURLs on their websites. One problem is that what is put up on the EURL websites is not controlled by the EU so we have to be sure that what is uploaded follows certain standards, have been validated. It seems that this work may be started again.

Questions:
Stig Mellergaard: I think it will still take a while before this decision will be implemented.
Niels Jørgen Olesen: but it is stated in the legislation that this should have been done in 2009.
Birgit Oidtmann: Apparently there is not much interest concerning the fish legislation. I think it is important that the NRLs state that it is important that something is being done in this area.
Olga Haenen: It could be a good idea that all of us NRLs sign a letter of interest that is addressed to the Commission, asking that we need the work to be finalized so we have diagnostic manuals to work after.
Sven Bergmann: I would like us to think about we have different kind of productions between cyprinids and salmonids. Eg. It will take 8 weeks to drain a carp pond, and it is stated in the legislation that this should be done within 6 weeks.
Niels Jørgen Olesen: I think that one of the reasons for nobody really taking it seriously is that we somehow fall in between agriculture and fisheries.

POTENTIAL EMERGING DISEASE OUTBREAK IN SWEDISH RAINBOW TROUT FARM

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Abstract:
In autumn 2011, a Swedish fish farm of rainbow trout experienced an increased mortality in newly hatched larvae that originated from imported American triploid eggs. Live fish were sent to the NRL which excluded bacterial and viral causes. The histological findings from the fish were so severe that the farm was placed under restrictions while awaiting the results. Further investigations have revealed findings that indicate that they are likely caused by a parasite in the family *Sarcocystidae* - which have not previously been demonstrated in fish.

Minutes:
All the health certificates from the fish were in order. Damages were present in all fish in musculature. Transmission test was performed and in a couple of weeks mortality started in the test group and within 4 weeks all the fish were dead and only 1 fish in the control group had died. This was so severe that it was decided to destroy the fish on the farm. Movement restrictions has been put on the water area. No consistent bacterial infections were found and no viruses could be identified from the fish. Histological investigations show infiltration of small intracellular organisms of protozoan type in several organs consisting of apicomplexan cells of a coccidian type. Severe changes in muscle tissue including necrotic areas were described as well. Cytology demonstrated the presence of protozoan cells in blood and kidney smears. The symptoms have never been seen before in Swedish fish and the parasite has, so far, only been detected in one fish farm.

Questions:
Stig Mellergard: Was the infection trials also with triploids.
Anders Hellström: yes.
Torsten Snogdal Boutrup: Whether the infection came with the eggs or not, this highlights the value of disinfecting the eggs in a conscious manner.
Anders Hellström: if the infection came with the eggs it has to be vertical, as the disinfection was done.
Niels Jørgen Olesen: this raises the awareness of the emerging diseases and how we should react to them. It seems that this i a very pathogenic disease, as the disease could be transferred via cohabitation.
Sven Bergmann: We all know that everything is different in fish. Do you know if the parasite sporulate on the eggs.
Anders Hellström: I am not a parasitologist, but what they told me it has to be inside the eggs, but we have not looked after the parasite inside the eggs.

SESSION II: Emerging diseases

Chair: Dr. Birgit Oidtmann
Minutes: Dr. Niccolò Vendramin

GEOGRAPHIC DISTRIBUTION OF SAV IN NORWAY

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Abstract:
Pancreas disease (PD) is a disease in farmed salmonid fish caused by a salmonid alphavirus. PD has been known in Norway since the 1980s, and the causative agent was characterized as Salmonid alphavirus subtype 3 (SAV3) which has, so far, only been detected in Norway. From 1995, PD was only detected in Hordaland county, but it slowly spread south and north along the coast, causing substantial losses to the fish farming industry. Measures were implemented by the Food Safety Authorities, fish farmers and well boat owners to control the disease. As a result, PD has mainly been confined to the area south of Hustadvika in Møre and Romsdal county, with only sporadic outbreaks north of this zone. In the spring of 2011 the first case of PD caused by a different subtype, marine SAV2, was discovered. Following this outbreak, marine SAV2 have spread rapidly in an area previously free of PD, resulting in a PD epidemics in Romsdal, Nordmøre and Trøndelag. A research project funded by the The Norwegian Seafood Research Fund (FHF) has been initiated in order to generate more knowledge on the significance of the marine SAV2. Extensive screening and characterization of virus from PD outbreaks diagnosed between 2008 and 2012 have been performed. The results show non-overlapping endemic zones with SAV3 present in South West and Western Norway and marine SAV2 from Romsdal to North-Trøndelag. In addition, a small challenge trial with selected marine SAV2 and SAV3 isolates has been performed. Preliminary results show that the marine SAV2 causes characteristic PD pathology. Based on the new PD situation, a revised plan for the control of PD has been implemented by the Food Safety Authorities, in which PD caused by SAV3 and the marine SAV2 are considered to be separate epidemics with different control strategies.

Minutes:
SAV causes Sleeping Disease in Rainbow trout, and Pancreas Disease in Atlantic salmon. Histo-pathological lesions are generally characterized by necrosis of pancreas and muscle. Vertical transmission is not recorded, horizontal transmission has been demonstrated. Apparently there is no transmission through vector (even though isolated from salmon lice). Spread from infected farm to one in close proximity.
It is a single stranded +RNA virus there are 2 orf; 1 for structural protein and 1 for non structural.
Six subtypes described on basis of genetic analysis.
Sub type 2 – France and UK – Rainbow trout, Scotland and Norway in Atlanticsalmon
Subtype 3 – PD Norway
In last 30 years only SAV 3 has been described in Norway!

Until 2003 few outbreaks, all located in the region of Hordaland.
From 2003 the outbreak number increased spreading north and south of this region. In the North of Norway only sporadical outbreaks related to movement of fish. No measures implemented in this period.
In 2007 the number of outbreaks almost doubled (from 58 outbreak to 98). The disease has become notifiable and action plan call PD-FREE consisting of Vaccination plus restriction of movement has been started in order to stop the spread.
The disease is compartmentalized in two main areas: South of Hustadvika PD is endemic, north PD free.
In April 2011 PD was diagnosed north of Hustadvika, virus characterized and SAV2 clustering with Scottish isolate.
A one year project funded from Norway in order to characterize all PD outbreaks from 2008, selection of isolated within the endemic Zone. The characterization of isolates demonstrated that first SAV2 was diagnosed during 2010 (occasional detection, no outbreak).
In 2012 46 detections occurred of SAV2 in the Hustadvika region. No stamping out was applied, in 2010 because it was considered SAV3.
SAV-2 caused low mortality and possibly subclinical infection.
Two SAV endemic zones, SAV-3 is south of Hustadvika, SAV 2 is north and this strain has somehow displaced SAV 3 as no SAV3 detection occurred since detection of SAV2. The two counties north of Hustadvika are SAV2 endemic and SAV surveillance areas, respectively. Farms in these counties are screened every month for SAV
.

Questions:
No question

VIRAL ENCEPHALOPATHY AND RETINOPATHY: THE FIRST VIRAL THREAT FOR MEDITERRANEAN FISH

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Abstract:
In 2011, total aquaculture production of sea bass and sea bream in the Mediterranean sea was estimated at around 277,000 tons, with sea bass alone representing 45% of the production with 126,000 tons (source APROMAR).
Viral Encephalopathy and Retinopathy (VER), otherwise known as Viral Nervous Necrosis (VNN) is caused by RNA viruses belonging to the Nodaviridae family, genus *Betanodavirus*. The disease, described in more than 50 fish species worldwide, is considered as the most serious viral threat affecting sea bass and other marine farmed species in the Mediterranean region. As a matter of fact, VER can cause devastating disease in the hatchery -inducing up to 100% mortalities- and a high mortality rate in farms, especially if associated to other pathogens (i.e. *Vibrio* spp., *A. ocellatum*). Indeed, this represents a major constraint for fish farming sustainability.
VER seems to be a considerable threat also for wild species, in particular for grouper and *Serranidae* species such as marble grouper (*E. marginatus*) and golden grouper (*E. costae*). Repeated mortalities in these species have been described in the wild (1).
Since 2008 a surveillance for the betanodavirus in the Ionian Sea has been performed by IZSVe with the collection of more than 600 wild fish samples. Fish were caught during scuba diving excursions and with experimental fishnets close to sea cages of a local sea bass/sea bream farm, in its surroundings as well as in some faraway areas. Surveillance was performed by real time RT-PCR and virus isolation. Several fish were found to be positive with different prevalence according to the sampled species and the diagnostic method. The possible interactions between the epizootics in farming sites and those in the wild are not fully understood and deserve further investigation.

References:
1) Vendramin N, Patarnello P, Toffan A, Panzarin V, Cappellozza E, Tedesco P, Terlizzi A, Terregino C, Cattoli G. (2013) Viral Encephalopathy and Retinopathy in groupers (*Epinephelus sp.*) in southern Italy: a threat for wild endangered species? BMC Veterinary research 9:20

Minutes:
A chapter on VER was reintroduced in the 2012 OIE aquatic diagnostic manual , but it is still NOT a listed disease.
Redspotted Grouper Nervous Necrosis Virus (RGNNV) is the most common, high water temperatures are required for development of outbreaks. Pathogenesis in the wild is not well known. There is a high risk for disease transmission from wild to farmed and vice versa. A lot of wild fish are attracted to sea cages as they are POI (??).
In 2007 a new farm was established in a naïve site. In 2008 a severe outbreak of VER occurred, 30% of losses disease over the year. Sanitary screening of fish wild around and in the cage started.
Fish were collected during diving excursion, providing a lot of information on the fish collected, and with trawling.
These samples were tested with histology, viral isolation (SSN-1 and E11 at 20°C and 25°C), and they were used for Real time PCR development.
3 sampling areas, close to the farm, MPA in Portocesareo and MPA of Torre Guaceto.

From 2008 a total of 627 samples (502 wild, 125 farmed), belonging to 56 different species were collected.
Most important sea bass positive, Wrasse, and Grouper. Different species of labridae tested positive. Also organisms different from fish demonstrated to be vector of the viruses.
Wild Grouper mortality occurred all data are in the paper.
Pathogenesis need to be understood better, in the sampling area Noda is quite widespread in the sampling area. It is difficult to detect wild diseased fish (only big ones). In wildlife latent infection can develop into clinical outbreaks under stressful conditions (in the case of grouper: unexpected high water temperature). Positive specimens detected might act as reservoirs. Still need to be clarified persistence time, and flow of viruses and wild fish.

Questions:
Sven Bergmann: Did you perform histology on molluscs?
Anna Toffan: No only on fish.
Snjezana Zrnčić: What about the finding of noda in artemia?
Anna Toffan: We detect it in a commercial batch, commercial artemia can be source of infection.
Snjezana Zrnčić What about gonads as sample to be tested?
Anna Toffan: We detected few positive samples on gonads, brain is the main target organ. Some positive samples were detected also in the Adriatic Sea but the majority of positive were in Ionian).
Athanasios Prapas. Could be SJNNV genotype be the cause the mortality in sea bream?
Anna Toffan: We had only outbreak in gilthead sea bream and it was RGNNV.

HIRRV, A NEW CANDIDATE FOR LISTED DISEASES

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Abstract:
In April 2007, a high mortality (roughly 80–90%) affected a grayling farm in the south of Poland at a water temperature of about 11–13°C. Adult grayling showed clinical signs characteristic of a viral infection, that is, petechial haemorrhages. Several weeks later, in June, significant mortality was observed in brown trout farm in the same region of Poland, approximately 120 km from previously affected farm. These brown trout exhibited abnormal swimming behaviour (spiraling, lethargy) and darkening of the skin. Stocking material was obtained from earlier infected grayling farm. In both of described cases HIRRV rhabdovirus was isolated on cell culture. Occurrence of the HIRRV was confirmed using the following methods: electron microscopy (NVRI, Poland), SISPA amplification (ANSES, France), RT-PCR (ANSES, NVRI), Real-Time PCR (ANSES, NVRI), serum neutralisation test (EURL, Denmark), IFAT staining (EURL, Denmark) and described.
The identification of HIRRV in two European trout farms can be considered as a emerging disease according to hypothesised relationships between anthropogenic processes and types of disease emergence in freshwater aquatic animals presented by Peeler and Feist 2011.
Theoretically the case of occurrence of HIRRV fulfills the criteria defined by Council Directive 2006/88/EC to apply on non-exotic listing diseases, but since 2007 no cases of HIRRV infection were confirmed in Poland.

References:

1) Peeler E. J., Feist S. W. (2011): Human intervention in freshwater ecosystems drives disease emergence. Freshwater Biology, 56, 705-716.

2) Borzym E., Matras M., Maj J., Baud M., De Boisséson C., Talbi C., Olesen N.J., Bigarré L. First isolation of hirame rhabdovirus (HIRRV) in freshwater fish in Europe, accepted on March 2013 by Journal of Fish Disease.

Minutes:
In 2007 2 farms showed abnormal and unexplained mortality; these two farms that was distanced 120 km apart displayed 90 % mortality in grayling and 50% mortality in brown trout.
Samples analysed tested positive in cell culture, but negative by ELISA for VHSV, IHNV SVCV and IPNV, electron microscopy performed on cell culture supernatant show classical rhabdivirus shape.
It was possible to neutralize the virus with anti-HirameRhabdovirus serum.
The origin of the infection could have been frozen fish imported from China and used for feeding.
A mortality outbreak in wild grayling occurred downstream to the farm but unfortunately no sample were collected and tested.
After challenge trials in both rainbow trout and grayling it was possible to re-isolate the aetiological agent and increased mortality was observed in grayling.
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Questions:
Sven Bergmann Which cell lines did you used to isolate the virus?
Marek Matras: We used BF-2, EPC, FHM and RTG-2.
Richard Paley: Did you perform surveillance?
Marek Matras: Until 2010 all farms provided samples every year but no detection at all.
Stig Mellergaard: Did you remember which fish species was frozen as feed?
Marek Matras: No data available, on fish species, origin, and area. Fish from Asia.
Vlasta Jencic: Do you rear fish for repopulating?
Marek Matras: Yes fish anglers association have project.
Vlasta Jencic: Is it usual to feed fish for repopulation with frozen fish?
Marek Matras: This practice is not employed very often.
Heike Shütze: Strange that the mortality was at low water temperature. Do you have information about the strain compared to the Asian one?
Marek Matras: The sequencing revealed high similarity with china isolate. The temperature was 11 degrees.
Niels Jørgen Olesen: Last year Dr. Hong Liu showed that this virus was detected in fresh water all over Northern China that is also a cold area. This seem to be a new disease introduced into Europe. There is the need to look into the wild.

ROUND TABLE DISCUSSIONS ON HOW TO DEAL WITH EMERGING DISEASES – DIFFERENT STRATEGIES AND APPROACHES

chair: Birgit Oidtmann

Minutes:
The target of this round table discussion was emerging diseases and how to address them.
The key points and highlights discussed are hereunder reported:

1) Emerging disease definition.

According to the OIE definition present in the OIE Aquatic Animal Health Code it is a "newly recognized infection resulting from the evolution or change of an existing pathogenic agent, a known infection spreading to a new geographic area or population or a previously unrecognized pathogenic agent or a disease diagnosed for the first time and which has significant impact on aquatic animal or public health.

2) The second point addressed was which possibilities are available to monitor the emergence of diseases:

Among these were cited www.AquaticHealth.net and a database available in CEFAS laboratory for emerging disease. It was underlined also that in a lot of countries there are different unofficial way to monitor the aquatic animal health situation and the occurrence of emerging and re-emerging pathogen.

- In Germany every second year an official meeting is held inviting expert from Austria Germany and Switzerland.
- In Norway there is an official system, the need to have someone belonging to an official body responsible for this task. It is remarked as well that these kind of system should have a predictive value and not only certifying that a disease has emerged once it has become a real problem. The case of PD was cited and now great focus should be given to EMS in shrimp.
- Britt Bang Jensen underlined the presence of a call last year to recognize the database in place and summarize them.
- In Italy there is an annual meeting with regional laboratories to keep updated all relevant colleagues involved in fish disease diagnosis, for the last three years there has also been an itinerant training addressed to veterinary officers.
- In Sweden there is an official on board of agriculture. The importance to look into the wild population was stressed.

3) The third point addressed was: When the finding/emergence of a disease/pathogen should be shared?

- Stig Mellergaard: the emergent disease should be listed when is still emergent not necessarily once has emerged. One important topic strictly related to emerging disease is the trading connections. Unexplained mortalities and uncontrolled trading connection will spread the disease extremely fast (i.e. Oyster herpes virus).
- Britt Bang Jensen: one of the points to address is how to deal with emergency plan for unexplained mortality.
- Olga Haenen: where you should go if you recognize a bacteria or a parasite as emerging pathogen?
- Birgit you should go to OIE and EU. Go to competent authority.

4) The fourth point stressed was that currently there is no formalized process on how to denounce an emerging disease. How to recognize an emerging disease at a national level.

- Stig Mellergaard: There is reluctancy to do it (look Bonamia), trade implications are obviously an important issue.
- Brit Hjeltens: Identifying risk factors and handling the risks is very important. In order to be able to spot some risks, the OIE provides some guidelines. It is very important that once a risk is highlighted it is referred to competent authorities.

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

Chair: Dr. Brit Hjeltnes
Minutes: Dr. Morten Sichlau Brunn

HEALTH CATEGORISATION OF FISH FARMS IN EUROPE IN 2012

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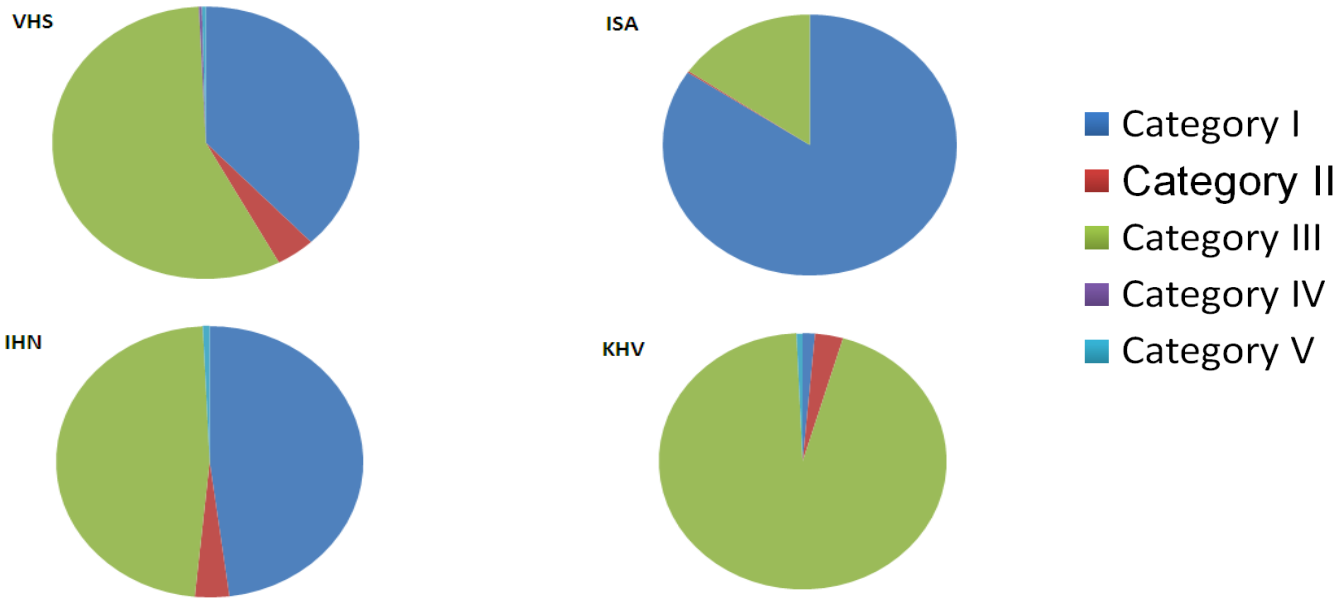
Abstract:
The Questionnaire on Surveillance and Diagnosis (S&D) included questions on how fish farms are health categorised according to Council Directive 2006/88/EC in the respective countries. Many countries provided very clear and correct answers but unfortunately a few more countries did not reply to the questionnaire when compared to previous. It is therefore not yet possible to obtain a complete overview of the status of fish health categorization in Europe.
More than half of the authorised farms in Europe are situated in category III zones for VHS and approximately half for IHN. For both diseases the remaining farms are situated in category I or II. Very few farms are placed in category V infected areas, and it is obvious that the diseases are very underreported. In all countries except Norway almost all salmonid farms are in Category I. Only very few carp farms are approved KHV free in Category I as almost all are placed in Category III.
Many farms in Europe are still not categorised, and unfortunately the situation have not improved much from 2011. There are several different views on how categorisation shall be performed, e.g. should VHS free marine rainbow trout farms be placed in Category III or I? If Isavirus HPR0 is found in or in proximity of a farm can it remain its Category I status? Some Member states do not include registered APBs in the categorisation but according to 2006/88/EC Annex III health categorisation comprise all APBs in the categories of the Member states, zones and compartments. A new Animal Health Law is under preparation and revision and will include aquatic animals; in this connection the categorisation system might be simplified and be made more transparent.



Minutes:

This presentation aims to provide a summary of the health categorization of fish farms that was started 3-4 years ago in Europe.
Authorization and categorization was in good process but since 2011 the process almost stopped in several countries and only very little progress has been seen since then.
Health categorization applies to ZONES and COMPARTMENTS (regions) and account to ALL Aquaculture Production Businesses (APB) in these both authorized and registered.
Different views are seen across Europe on how categorization shall be performed: e.g. regarding the marine/freshwater question for VHS and categorization of ISA in I or III
As very few farms in Europe are declared infected (Cat V farms) this is either a case of almost no VHS and IHN infected farms in Europe, or that many *de facto* infected farms are being reported as Cat III? It is most likely that the diseases are much underreported.
Many farms (6-8000) are placed in Cat III and have to be inspected by active surveillance according to risk ranking (Annex III)
The same surveillance applies to the many farms in Cat.II, and it is a question whether all these surveillance programs are informed to or approved by the Commission.
The situation in individual countries was not presented, but can be found in the presentation.

Questions:
Brit Hjeltnes: What are the consequences of the "accumulation" of Cat III farms?
Niels Jørgen Olesen: It is a difficult question as the Cat III has become a "rag-bag". Ideally, farms should move to Cat I or Cat V within years, but the opposite has happened. Perhaps because the industry doesn't want to be stigmatized as infected?

RISK BASED SURVEILLANCE IN AQUACULTURE: THE OUTCOMES OF AN EFSA PROJECT

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Abstract:
The objective of this project was to describe and critically assess the factors necessary to reliably and efficiently categorise fish farms taking into account characteristics of the diseases listed in Part II of Annex IV of the Council Directive 2006/88/EC. Such work should provide the basis for the development of risk-based surveillance in European Member States (MS) using a standardised approach.
The project was structured into 6 tasks:
1. Perform a literature review of risk-based surveillance methods for farmed/wild and terrestrial/ aquatic animal surveillance.
2. Describe the present level of implementation of the Article 10 of Council Directive 2006/88/EC provisions on risk based surveillance and surveillance for demonstration of disease freedom of fish diseases.
3. Describe the characteristics of the listed fish diseases that may be relevant for the development of risk-based surveillance schemes. Provide a scientific justification for the selection of these characteristics and the proposed risk classification.
4. Describe and assess the various factors necessary to categorize fish farms in Europe taking also in account the results of 3.
5. Develop a methodology for ranking of fish farms using disease specific characteristics and farm categorization in order to develop risk based surveillance for demonstration of disease freedom
6. Undertake case studies to validate the output of the risk categorization and ranking for the development of a risk based surveillance scheme for a fish disease which is exotic to the EU and a disease which is present in a limited number of Member states.

An overview of the project will be presented. The developed model calculates a quantitative risk score for individual aquaculture sites. The final calculated risk score is a value between zero and one and is intended to indicate the risk of a site relative to the risk of other sites (thereby allowing ranking). The model is suited for assessment of individual fish farms to rank farms to support surveillance to demonstrate disease freedom.
The work was undertaken in a cooperation Art 36 project "Risk categorization for Aquatic Animal Health surveillance" (CFP/EFSA/AHAW/2011/03) of the European Food Safety Authority (EFSA).

Minutes:
An overview of an EFSA-project on risk based surveillance in aquaculture that finished in February 2013 was presented. The project involved ranking of fish farms based on demonstration of disease freedom (in contrast the legislation is based on presence of disease).
Papers and EAFP presentations of the results are in the pipeline.
An overview of the whole project and more specifically the 6 tasks was presented:
Task 1, literature review: Several examples of risk based surveillance in terrestrial animals were identified, but examples were largely absent in aquatic animals.
Task 2, the present level of implementation: The results from a questionnaire were presented by Brit Bang Jensen at the Annual Meeting in 2012. Implementation is still in progress.
Task 3, risk characterization of fish farms: As a result of a brain storm on which direction the model should go, a paper was published in 2011 (Pathogen introduction and spread) that identified 5 risk themes: 1) Live fish and egg movement, 2) Exposure via water, 3) On-site processing, 4) Short distance mechanical transmission, and 5) Distance independent mechanical transmission.
These principal pathways were subsequently confirmed.
Task 4, factors necessary to categorize fish farms in Europe: Additional experts were involved (via supplied spreadsheets and subsequent discussions) to examine if fish farms in a given country can be grouped into risk categories suitable to inform risk based surveillance. The results showed that in general there can be difficulty in obtaining data, and different approaches to categorization were seen. The conclusion of this task was that farms have to be risk categorized individually.
Task 5, risk based surveillance to demonstrate disease freedom.
Task 6, case studies: Huge range of risk scores (using expert factors) were seen in VHS case studies using data from England (VHS free) and Italy (VHS history) as well as few data from Switzerland. Risk scores ranging from close to zero to 0,78 was seen.

Questions:
No questions.

THE ANIMAL HEALTH LAW FROM AN AQUATIC PERSPECTIVE

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Abstract:
The proposal for the new Animal Health Law is an attempt to simplify and streamline the EU legislation on animal health both for the terrestrial and for aquatic animal health

The new Animal Health Law is a piece of framework legislation which at a later stage will be supplemented with secondary legislation.

A review of the proposal will be presented with special emphasis on the issues that may be of interest and importance for aquatic animal health.

Minutes:
A step by step introduction to the new Animal Health Law (AHL) was presented with focus on news and the aquaculture perspective. The law was released 2 weeks ago.
AHL is a framework legislation and will be covered by supplements later. It replaces 31 directives and regulations (supported by more than 400 pieces of secondary legislation).
The regulation lays down rules for the prevention and control of animal diseases, which are transmissible to animals or/and humans (the mention of humans here is NEW).
Part I covers general rules, and again mention of "impact on human health and medical treatments" are new additions as well as "the zoonotic character of the disease", "the capacity to develop resistance to treatments" and "Potential to generate a crisis situation and its potential use in bio-terrorism". Categories of listed diseases were presented (article 8).
The responsibilities for animal health were defined.
Part II includes early detection, notification and reporting of diseases, surveillance, eradication programmes and disease-free status. A new addition to surveillance is "Animal health visits"
Part III concerns disease awareness, preparedness and control, and includes contingency plans, simulation exercises, the use of veterinary medicinal products for disease prevention and control, antigen, vaccine and diagnostic reagent banks, and disease control measures.
Part IV includes registration, approval, movements and traceability of both terrestrial animals and specifically aquatic animals and products of animal origin from aquatic animals: Registration, approval, record keeping and registers as well as movements within the Union of aquatic animals other than aquatic pet animals, movements within the Union of aquatic pet animals, production, processing and distribution within the Union of products of animal origin from aquatic animals, other than live aquatic animals, and national measures including prevention and control of non-listed diseases.
Part V covers entry of consignments of animals, germinal products, and products of animal origin into the Union and the export of such consignments from the Union.
Part VI lists emergency measures to be taken in the event of a disease emergency situation.
The future secondary legislation will be essential, and the Lisbon Treaty will establish the framework for the elaboration and adoption of secondary legislation
Delegated acts – non-essential elements: Commission have the power to issue new legislation – followed by scrutiny by EP, and if necessary experts may be consulted.
Implementing acts shall be approved by MS via SCOFCAH.

Questions:
Olga Haenen: Will diagnostic manuals be part of the secondary regulation?
Stig Mellergaard/Niels Jørgen Olesen: The diagnostic manuals are the ”next” level.
Niels Jørgen Olesen: Will the forthcoming secondary legislation be divided into aquatic and terrestrial animals, or will these animals be regulated together?
Stig Mellergaard: There will be both this condition depending on the topic.
Henrik Korsholm: Where can details about test frequencies etc. be found?
Stig Mellergaard: This will be part of the secondary regulation.

HOW TO DEAL WITH EPIZOOTIC ULCERATIVE SYNDROME AFTER ITS DELISTING

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Abstract:
Under EU Directive 2006/88, Epizootic Ulcerative Syndrome (EUS) was listed for the first time as an exotic disease to Europe and became notifiable. The listing of the disease lead to a range of challenges: e.g. diagnostic capability in national reference laboratories needed to be developed, and the evidence for freedom from EUS may have been challenged internationally if put to the test.
On request of the EU Commission, EFSA provided a scientific opinion on the risk of introduction of EUS into EU aquaculture and the potential impact on EU aquaculture (published in 2011). It was concluded that the majority of fish species reared in EU aquaculture were not highly susceptible to EUS and therefore the impact of EUS on aquaculture in Europe (if introduced) was likely to be relatively low. It was recognised that there were insufficient data from monitoring of aquaculture or wild stocks in Europe to say whether EUS may have been introduced wild or aquaculture populations in Europe in the past. It was also recognised that pathogens could be released from so called 'closed ornamental facilities' through the sale of fish to hobbyists, insufficient effluent treatment or unintentional contact with natural waters. Therefore, *Aphanomyces invadans* may have been released into the aquatic environment in Europe in the past and has either not lead to establishment in fish populations, or has not been diagnosed.
Based on the conclusion that – if introduced – the impact of EUS on aquaculture in the EU is likely to be low, EUS was removed from the list of notifiable diseases under Council Directive 2006/88 at the end of 2012.
The EFSA opinion recommended that scientifically based surveys for aquaculture, live imports and wild fish should be considered. This would provide evidence on the actual situation with regards to EUS in the EU. Furthermore, it was recommended that diagnostic methods suitable to detect *A. invadans* should be applied when clinical signs consistent with EUS are observed in fish.

In 2012, OIE (World Organisation for Animal Health) received a request to revise whether EUS should remain an OIE listed disease. In response, OIE set up an ad hoc group, which assessed whether EUS fulfilled the OIE criteria for listing of a disease. The ad hoc group concluded that EUS fulfilled the criteria and as a result, EUS remains an OIE listed disease.

Minutes:
Epizootic Ulcerative Syndrome (EUS) was first listed in EU under Directive 2006/88, where EUS was declared an exotic disease. As EUS never has been reported in EU, EFSA was asked for a scientific opinion on the risk of introduction, spread, persistence and impact of EUS in EU. EFSA concluded that the impact of EUS on aquaculture in Europe (if introduced) was likely to be relatively low. EFSA did not assess the effect/impact on wild fish. EUS was subsequently de-listed in 2012 in EU.
An OIE (World Organisation for Animal Health) ad hoc group on listing of EUS reached a different conclusion in September 2012, and found that EUS fulfilled the OIE criteria for listing of a disease and meets the requirements for listing, and EUS thus remains an OIE listed disease. .
Mainly countries with high summer temperatures should be concerned.

In relation to EUS, mortalities in wild fish could be important, and perhaps misdiagnosis happen as mortalities often are attributed to poor water quality and *Aeromonas hydrophila*. The right samples need to be taken and investigated. But who is in charge of investigating mortalities in wild fish?
A review paper on EUS is being published.
The presentation was ended with an appeal for all to take samples and look for EUS.

Questions:
Perttu Johannes Koski: How should it be handled if a third-country asks for EUS-free fish from EU aquaculture?
Birgit Oidtmann: It is a problem – how many fish to sample? Especially in low susceptible fish species as Rainbow Trout.

SESSION IV: Scientific research update

Chair: Dr. Richard Paley
Minutes: Dr. Niccolò Vendramin and Dr. Morten Sichlau Brunn

MOLTRAQ - MOLECULAR TRACING AND EPIDEMIOLOGY OF VIRAL DISEASES IN AQUACULTURE

Susie Sommer Mikkelsen[1], Britt B. Jensen[2], Peter A. Jansen[2], Niels Jørgen Olesen[1], Helle Frank Skall Laurent[1], Bigarré[3], Heike Schütze[4], Svenn M. Bergmann[4], Tristan Renault[5], Jean-Christophe Avarre[6], Magne Aldrin[7] and Edgar Brun[2]

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6Norwegian computing center, Oslo, Norway

Abstract:
Here we present a new research-project funded under the EMIDA-ERA Net under the EU 7th Framework program. For more details on MOLTRAQ, go to www.moltraq.wordpress.com
European aquaculture production of both fish and molluscs are constantly under threat of infection by devastating viral diseases. Some of the most significant viral families include the Alphaviridae, Rhabdoviridae, Betanodaviridae, Malacoviridae and the Alloherpesviridae.
Surveillance and control of pathogens is a common strategy for combatting the spread of viral diseases in aquaculture. Often this control is carried out on the basis of general knowledge of bio-security and fails to take into account disease-specific transmission patterns, of which little is known. Therefore, knowledge of the factors that regulate the viral spread and pathogenesis are of high importance to be able to predict outbreaks and design efficient control strategies.
A core goal of the MOLTRAQ project is to generate and use spatio-temporal epidemiological data, phylogeographic data and gene expression data for important host-viral pathogen systems to identify important factors affecting the spread of diseases in aquaculture. These data will then be used to establish generic modeling tools for exploring effects of different intervention strategies for selected host-pathogen systems. Furthermore, phylogenetic studies will be undertaken and genotypes of particular interest for gene expression studies will be selected for investigation of molecular markers. Oligonucleotide microarrays will be developed to study the changes in virulence and gene expression according to temperature.
All epidemiological and genetic data on isolates of the viruses in this project will be uploaded in the isolate database www.fishpathogens.eu. A new SAV database will also be developed and published.
MOLTRAQ aims to collect and collate new data on several of the most important European aquatic pathogens and use these data to generate a better understanding of the spread of pathogens in aquatic environments.

Minutes:
An update on MOLTRAQ was presented, as the project was introduced by Brit Bang Jensen at the Annual Meeting 2012. Six different institutes and two additional partners are involved, but the group is open to additional collaboration (voluntary).
The six work packages were explained, and the following details were presented:
WP2, Collection of virus sequences and epidemiological data:

Collected viruses: Herpesviruses (OsHV-1, KHV/CyHV3), Rhabdoviruses (VHSV, IHNV , percirhabdo, HIRRV, EVEX), Nodaviruses, Salmonid alphaviruses (SAV), Birnaviruses (IPN).
Positive samples of OsHV-1 from molluscs have been collected from France (approx. 400) and other countries (50) from 1993 -2012. At least 15 full-genome OsHV-1 will be sequenced by IFREMER and partial sequencing of specific regions with areas of interest will be done on collection of isolates from different countries.
Numerous VHSV isolates from several countries are included and will be sequenced and supplemented with already published data resulting in almost 600 full length g-gene sequences.
The sequences obtained in MOLTRAQ and already published data will be used in WP3 concerning phylogeny and evolution of the viruses.
Information can be found on www.fishpathogens.eu. Work on SAV-database is starting.
WP4 concerns effect of temperature on gene expression patterns. The role of temperature in triggering virulence will be investigated either by microarrays or high throughput qPCR.
WP5, Scenario simulation models for control options: Mathematical models for intervention strategies will be developed for VHSV and perhaps OsHV.
More information can be found at www.moltraq.wordpress.com.

Questions:
Olga Haenen: Will the work start with VHSV and then include other virus at a later stage?
Susie Sommer Mikkelsen: No, all virus groups at the same time.
Olga Haenen: We are interested in collaboration.
Brit Bang Jensen: Two MOLTRAQ presentations on respectively OsHV and KHV as well as a poster about the project will be present at the next EAFP conference.
Niels Jørgen Olesen: We are planning on organizing workshops – maybe in connection with Annual meeting EURL.

PHYLOGENETIC ANALYSIS IN FISH DISEASES

Heike Schütze[1]

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The hands-out version of this presentation is attached as annex.

Minutes:
An overview of phylogenetic analysis was presented, including features as molecular analysis, morphology and biochemistry. The advantages of molecular analysis are the huge amount of comparative features.
As there is no way to measure whether a particular phylogenetic hypothesis is accurate or not the best result is obtained with a tree with branches that are well supported by the available evidence.
In addition, inclusion of more taxa results in a more accurate analysis and thus more robust results of phylogenetic tree. Phylogenetic analyses illustrate the development and evolution of the agent, whereas epidemiology can tell us something about the correlation between virus distribution and trade channel.
New software tools are available, e.g. SPREAD, that can combine genetic and epidemiological data. There is a need to have scientists to evaluate the output.

Questions:
Richard Paley: What is the status on sequencing isolates from the EU proficiency tests?
Niels Jørgen Olesen: All laboratories in EU perform perfectly now in the proficiency test.
There has been a lot of progress in 10 years.

ATLANTIC HERRING SHOWS HIGH MORTALITY RATE IN BATH CHALLENGE WITH VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS (VHSV)

Renate Johansen[1], Torsten Snogdal Boutrup[2*], Helle Frank Skall[2], Nina Sandlund[3], Britt Gjerset[1], Ingebjørg Modahl[1], Øivind Bergh[3], Niels Jørgen Olesen[2]
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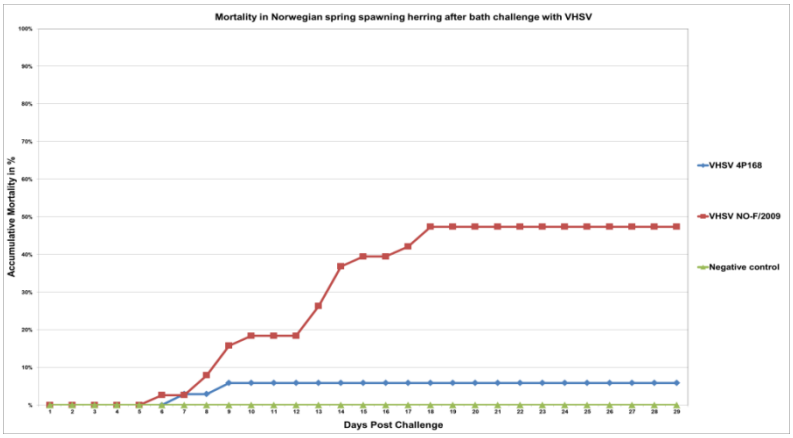
Abstract:
Earlier investigations have demonstrated the presence of viral haemorrhagic septicaemia virus (VHSV) in European herring *Clupea harengus* in the North Sea, Baltic Sea, English Channel, Skagerrak and Kattegat. Most of the approximately 100 VHSV isolates included in the www.fishpathogen.eu database are of genotype Ib, four are of genotype II and one is of genotype III (4P-168). VHSV is also well known in Pacific herring *Clupea pallasii* where genotype IV have been detected both in diseased and healthy wild herring. Challenge trials with genotype IV isolates in Pacific herring in the US have shown high mortality rates, while no VHS challenge trials have earlier been conducted on European herring.
High prevalence of VHSV genotype Ib was detected in Norwegian spring spawning herring during the spawning season (Johansen et al 2013). The sampled herring showed no signs of disease and were caught by commercial trawl boats. High amounts of virus was found in internal organs (heart, kidney, spleen and brain) showing a septicaemia. How this virus affects the herring stock is unknown. A bath challenge trial with one of these isolates from Norway (NO-F/2009) was conducted on Atlantic herring of approximately 3 gram. In addition herring was challenged with a genotype III isolate from herring (4p168). The Norwegian Ib isolate from herring gave an accumulated mortality of 47% compared to 6% morality with the genotype III isolate. Results from histopathology, immunohistochem., RT-PCR and cell culture will be presented.How severely VHSV affects wild stocks of herring in the Northern European waters, and which threat VHSV in wild fish represents to farmed fish are two of the main questions that need to be further investigated.



Reference:
Johansen et al. 2013 "High prevalence og viral haemorrhagic septicaemia virus (VHSV) in Norwegian spring-spawning herring" Marine Ecology Progress Series 478: 223-230

Minutes:
A Norwegian/Danish collaboration project on VHSV in herring was presented.
Mainly genotype Ib has been isolated from wild Herring (33% VHSV prevalence in spawning herring), and has been used in this project. Infection trials in USA with North American herring have shown mortality. Some of the challenges in keeping wild herring for trial were presented: Special net, transport of Herring, aquarium design etc. Maintaining a robust environment is perhaps

the most critical factor. When working with wild caught fish it can be necessary to intervene using e.g. formaldehyde for parasites and antibiotics for bacterial problems.
The challenge resulted in approx. 50 % mortality with Norwegian VHSV strain, and only 6% mortality with a Danish genotype III. The gross pathology included bleeding in the mouth. PCR analysis showed single positive samples on day 4-5 PI for both virus isolates, increasingly more positive samples were detected until day 20 after which a decreasing number of positive samples was seen. Apparently the heart is target organ as more hearts than kidney and gills are positive by PCR and heart also shows lower CT values. Results from immune-histochemistry were presented. At termination day 30 PI virus was re-isolated from both groups on BF-2 cells.
Importance to herring: Genotype III – not high mortality, genotype Ib – high mortality, and may have an effect on wild herring populations? Importance to Rainbow Trout: Low mortality, but we need more information on virulence factors.

Questions:
Birgit Oidtman: Remember a wild Herring publication: outbreak of VHS-disease when stressed in tanks.
Torsten Boutrup: Herring from the southern part of Denmark were used – free of VHS. 250 fish were tested by PCR and culture without any VHS findings.
Niels Lorenzen: Importance in wild population?
Torsten Boutrup: Perhaps a normal part of the ecology, but perhaps VHS could be a factor in sudden high mortality in wild Herring?
Henrik Korsholm: Antibody tests?
Torsten Boutrup: No.
Brit Hjeltnes: Herring stocks recent years have been big – relevance? Did you found them positive for skin lumps?
Torsten Boutrup: Haven't found *Ichtyophonus* (skin lumps)

MOLECULAR FEATURES OF LOW AND HIGH PATHOGENIC CLONES OF VHSV IB ISOLATES

Takafumi Ito[1], Jun Kurita[1†], Koh-ichiro Mori [2], Helle Frank Skall[3], Niels Lorenzen[3], Katja Einer-Jensen[3‡], Niccolò Vendramin[3], and Niels Jørgen Olesen[3]

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Abstract:
VHSV Genotype specific mAbs were produced in order to establish a fast and low cost genotyping system for VHSV isolates (Ito et al. 2012). In this process, we interestingly encountered a number of exceptional reactions with various VHSV isolates. One mAb, VHS-3.80, that recognize all genotype (G) Ib, Ic, Id and II isolates, did not react with the GIb SE-SVA-14 isolate from diseased sea farmed rainbow trout *Oncorhynchus mykiss* from Sweden in 1999. No-reaction was also found for a variant clone (SE-SVA-1033-3F) that was a derivate from of a GIb isolate from another outbreak in Sweden in 2001. These 2 isolates from Sweden are till now the only GIb known to cause mortality in farmed rainbow trout. In contrast mAb VHS-4.20, which react with all VHSV genotype except GIb did react with a variant clone of the SE-SVA-14 isolate. Since GIb isolates in general are low virulent for rainbow trout, and as these Swedish isolates are virulent the atypical reactions of the clones could be related to virulence determinants of GIb in rainbow trout. Based on the results from a virulence testing of these clones in rainbow trout and in amago trout *Oncorhynchus masou macrostomus* by infection trials in Japan and Denmark, and based on a comparative analysis of the entire genome of these clones we suggest that the substitutions of amino acid in the nucleo-protein regions aa 43 to 48 and aa 168 could be such pathogenicity determinants;
The very limited genetic difference between low and high virulent VHSV variants implies that some low virulent VHSV GIb isolates in the marine environment could quite easily mutate into high virulent strains and this should be considered as a potential threat for the trout farming industry.

References

1) Ito,Takafumi, Jun Kurita, Motohiko Sano, Helle Frank Skall, Niels Lorenzen, Katja Einer-Jensen and Niels Jørgen Olesen (**2012**)Typing of viral hemorrhagic septicemia virus by monoclonal antibodies. Journal of General Virology, 93, 2546–2557

Minutes:
Work on molecular features of low and high pathogenic clones of VHSV Ib isolates was presented. During VHSV typing by monoclonal antibodies using IFAT in large panel, one isolate reacted unexpected. Further investigation showed it consisted of more than one type. Two isolates were cloned resulting in 4 different clones. Could this lead to tracing a pathogenicity factor? Sequencing

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and alignment was carried out. Infection trial showed high mortality in Rainbow Trout, but huge difference in susceptibility when testing *Oncorhynchus masou*. IP-trial showed high mortality whereas immersion showed no mortality. It was argued that this is a case of host-pathogen interaction, and that the virulence is a matter of both virus entrance to cell of host (G-protein related) and on virus propagation in the cell (N-protein related).In conclusion pathogenicity is a question of host-pathogen interaction, even closely related *Oncorhynchus* species like (*mykiss* and *masou*) respond differently.
Reverse genetics might help us to assess if these substitutions in the N-protein are sufficient to increase mortality in Rainbow trout significantly.
Virulence = Entrance (host defense) + propagation (cell level defense)

Questions:
Britt Bang Jensen: Recently OIE has started discussing if it is possible to distinguish between pathogenic and non - pathogenic strains, which are the possibilities in the case of VHSV?
Niels Jørgen Olesen: Good tools to differentiate today, but we cannot always link genotype to pathogenicity (+ species differences + mutations).
Niels Lorenzen: Did you perform Re-isolation of virus in non-diseased fish?
Niels Jørgen Olesen: Yes we did but as the fish were IP-challenge so the virus was introduced directly in the host.
Birgit Oidtman: What is the risk associated with processing of fish – liquid waste into rivers – risk of disease spread?
Niels Jørgen Olesen: I would consider it a very small risk.
Perttu Johannes Koski: Is it a deletion like ISA-virus?
Niels Jørgen Olesen: No – a substitution.
Sven M. Bergmann: If we talk about the virus we should address virulence factors, if we talk about the disease we should refer to pathogenicity factors.

MOLECULAR TRACING OF IHNV IN EUROPE

Heike Schütze[1]

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The hands-out version of this presentation is attached as annex.

Minutes:
Within the Moltraq project one of the task is to collect, sequence characterize and compare different IHNV strains provided by different laboratories in Europe, preliminary data show relation between isolates sequence from Germany, France, Italy and Switzerland.
Specifically referring to Germany between 94 and 97 all isolates were clustering together, after eradication measures no isolates of same cluster was seen for 15 years; during the last year a strain clustering very close to this old strains appeared again; it is hard now to give explanation.
There are few sequences available; when a complete panel of isolates and sequence will be available it will be possible to perform a better risk assessment.

Questions:
Birgit Oidtmann: Why few IHNV from the Atlantic Ocean?
Heike Shütze: I am not sure you will find IHNV in marine fish, and why info is so limited I don't know.
Niels Jørgen Olesen: IHNV has caused problem with farmed Atlantic Salmon in sea water. It is a high risk in Europe and it needs to be addressed in risk assessment. IHNV was introduced in 1987 in Europe
Heike Shütze It could be an issue regarding susceptible species
Brit Hjeltnes: If is proven that IHNV is pathogenic to Atlantic salmon specific it could be a genotype not present in Europe.
Olga Haenen we had asymptomatic cases in Rainbow trout in freshwater
Niels Lorenzen Do you know why we see huge differences in host species between VHSV and IHNV?
Heike Shütze one of the explanations could be a bias due to the fact that VHSV was a huge problem of Great Lakes, while IHNV is lacking of interest.
Helle frank Skall we have to remember that surveillance was performed mainly on BF-2 in the wild, that's not optimal for IHNV.

FISH AND MICRO-RNAS

Dennis Bela-Ong[1], Brian Dall Schyth[1] and Niels Lorenzen[1]

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Abstract:
Fish is an important small vertebrate multidisciplinary model for investigating various aspects of reproduction, development, disease (immunology, toxicology, carcinogenesis), and aging. It is also an important model for comparative and evolutionary studies because it represents the lower vertebrates and serves as an essential link to early vertebrate evolution. Microribonucleic acids (miRNAs) are 18-22 nucleotide-long endogenous RNAs that bind to specific mRNAs, usually at the 3'-untranslated region, (UTR), thereby potently regulating a wide spectrum of target mRNAs. This adds a new layer to the mechanisms of control of gene expression, impacting a broad range of biological processes. Thus far, >25, 000 miRNA sequences have been identified in 193 species, including fish. In fish, the interest on miRNAs started with the analysis of their expression and function during embryonic development. In our lab, we investigate miRNA regulation during viral infection and vaccination in rainbow trout. We aim to identify miRNA biomarkers during infection and vaccination in order to understand the complex web of interactions involved in the underlying host immune responses. They may also be used as suitable selection markers to identify disease-resistant fish.

Minutes:
Micro-RNA (miRNA) are small (18-22 nucleotides) non-coding RNA involved in control of gene expression through RNA interference. More than 20000 miRNAs have been identified in 193 species. miRNA can be highly conserved (eg. dre-miR-155 in zebra fish and hsa-miR-155 in humans). A searchable database of published miRNA sequences and annotation can be found at miRbase.org
miRNAs are associated with disease (cancer, immune function disorders, neurological disorders etc.) leading to development of miRNA-based therapeutics.
Identification of virusinduced miRNAs in fish with VHS has been done via micro-array and qPCR using liver. miR-462 and miR-731 are induced by interferon, and the effect is unknown – experiments are still ongoing to investigate a possible antiviral effect. In addition some virus can produce miRNA.
In-silico analysis of putative targets in the VHSv genome is being done.
Evaluation af potential function(s) of miRNA – treat cells & fish with miRNA/anti-miRNA.

Questions:
Birgit Oidtmann: how can we apply miRNA in aquaculture, future problem for consumers?
Denis Bela-Ong: no they are very volatile, easily degraded
Irene Øperveit: how to administer them?
Denis Bela-Ong: in fish IP injection is efficacious; in human intravenous injection has been used
Richard Paley: Did you find in VHSV target for miRNA
Denis Bela-Ong: some virus has genes encoding for miRNA that interact with host. We still don't know in the case of VHSV
Niels Jørgen Olesen: Which is the effect of interferon I in relation to miRNAs?

Denis Bela-Ong: it induces the production miRNAs, Interferon is induced during infection and interferon induce miRNA, so Interferon I influences and improves the response of interferon to viral infection.
Anna Toffan: did you test the direct effect of miRNA with virus?
Denis Bela-Ong: An experiment is still ongoing.

SPATIO-TEMPORAL RISK FACTORS FOR VIRAL HAEMORRHAGIC SEPTICAEMIA IN DANISH AQUACULTURE

B. Bang Jensen*[1], A.K. Ersbøll[2], H. Korsholm[3], H.F. Skall[4] and N.J. Olesen[4]

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Abstract:
Viral Haemorrhagic Septicaemia (VHS) is an economically very important fish disease in most of the world. When VHS virus was first isolated in Denmark 50 years ago, around 80% of the approximately 800 Danish fish farms were considered to be infected, but vigilant surveillance and stamping-out programmes have led to a drastic reduction in prevalence, and eventually a final eradication of VHS. The disease have now been absent in Denmark since 2009.
Data on outbreaks within the country has been collected throughout the years, and recently all farms have been geo-referenced. This has made it possible to use spatio-temporal scan statistical tools to search for clusters of high prevalence. Such tools have previously been applied on terrestrial animal diseases, but this is the first time they are used for aquatic animal diseases.
The analyses revealed a statistically significant cluster in the south-western part of the country, which persisted throughout the study period 1982-2008. Three additional spatio-temporal clusters in different time periods were also identified.
Further statistical analyses were performed on a subset of the farms;
A semi-univariable analysis showed that type of production (marine/freshwater) was not important for VHS, when accounting for whether the farm was situated inside a cluster of high risk.
A further analysis was performed on inland freshwater farms where the effect of year, number of farms in a stream and number of upstream farms on the probability of VHS was investigated. Being situated inside one of the identified clusters or not was also included as a risk factor. The variables; year, inside/outside a cluster and number of upstream farms were all significant risk factors for VHS ($p<0.001$).
The spatio-temporal scan tools provide an easy method for determining high-risk areas, also in aquaculture. Further, some important risk factors were identified. Both are valuable contributions when designing risk-based surveillance as required by the current European aquaculture animal health legislation. The Danish case is the first example of successful eradication of VHS in an endemic area.

Minutes:
An overview was presented of a survey achieved on the Danish register after the VHSV eradication has been achieved.
Thanks to the availability of an electronic register of every fish farm established in 1982 it was possible to link the farms to the water catchment, river and water source.
Data obtained with the survey plotted on Sat Scan can correlate the presence of the infection with space and time.
It was possible to target different risk areas, to demonstrate that the proximity of other infected farm is more relevant than water catchment in increasing the risk of contracting the infection.

A high number of farm in the water catchment increase the risk of contracting the infection no matter they are upstream or downstream.
The distance seems to be more relevant than the water source, for increasing the risk of contracting VHS.
It is not really relevant how many farm there are in the stream but how many farms that are situated upstream.
The satScan need to set data together with an expert of the data in order to obtain reliable results.
In the MOLTRAQ project the appearance of different genotypes correlated to the distance of farms will be investigated.
In conclusion the SatScan seem to be useful for risk-based surveillance

Questions:
Birgit Oidtmann: Did you look to odds ratios of contracting infection when an upstream infected farm was present?
Britt Bang Jensen: we will do it including genetic analysis to confirm that the farm is infected with the same strain.
Niels Jørgen Olesen: odds ratio will be 1, because one farm is infected all downstream will be infected.
Henrik Korsholm: I recall two cases where downstream farms did not get infected downstream, in one case the downstream farm was 28 km distant, the other the outflow was going into two big lakes so huge factor of dilution. Concerning the density of farms in the river upstream farms can get easily infected.
Torsten Snogdal Boutrup: I think it is nice model, but you need a lot of data to make the model, can you use it in another country, and how many data do you need?
Britt Bang Jensen: Data needed are where farms are and which one are infected, should be easy in future when legislation will be in place.

COHORT STUDY OF EFFECT OF VACCINATION ON PANCREAS DISEASE IN NORWEGIAN SALMON AQUACULTURE

Britt Bang Jensen[1], Anja B. Kristoffersen[1,2], Camilla Myr[3], Edgar Brun[1]

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Abstract:
Pancreas disease (PD) is an economically important viral disease in Norwegian aquaculture, with 75 to 89 annual outbreaks from 2009 to 2011. To hinder further spread of disease from an initial endemic area on the west coast of Norway, measures for surveillance and control are in place, and the disease is notifiable on a national level. Since 2008, the Norwegian coastline has been divided into 2 administrative zones separated by a production-free area of 10 nautical miles at approximately 63°N. At the same time, a vaccination program involving most marine salmonid farms was initiated by the industry, using a vaccine against PD that was made commercially available in 2007. The effects of the vaccine in the field have been questioned, since the annual number of PD outbreaks has not decreased as expected. However, other production parameters can be used for evaluation of vaccine effect, and in this study the effects of vaccination on cumulative mortality, growth rate, feed conversion factor and number of discarded fish were analyzed using data collected from fish cohorts with and without PD put to sea between spring 2007 and spring 2009. The results show that vaccination against PD has a positive effect in reducing the number of outbreaks, and decreasing cumulative mortality and the number of fish discarded at slaughter.

Minutes:
Refer to the entire paper:
http://www.int-res.com/abstracts/dao/v102/n1/p23-31/

Questions:
Niels Lorenzen: when you do things in experiments you have controls how could you evaluate the efficacy of the vaccine?
Britt Bang Jensen: The presence of the infection was considered and mortality rate was compared to cohorts in the same site.
Niels Lorenzen: Could you appreciate an addictive effect on mortality due to PD when IPN was present in the site?
Britt Bang Jensen: this was not significant.
Niccolò Vendramin: what about economic sustainability
Britt Bang Jensen: there is an old study showing that it is sustainable.
Athanasios Prapas: has MSD provided lab data? the RPS? 50-55%?
Brit Hjeltnes: they did, results are promising but results in the field can be different.

PROFISH: WHEN APPLIED VACCINE RESEARCH MEETS THE NEED OF THE INDUSTRY

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Abstract:
Despite vaccination with oil-adjuvanted vaccines against vibriosis and furunculosis, sea reared rainbow trout in Denmark often encounter outbreaks of furunculosis caused by *Aeromonas salmonicida* during warm summer periods. This implies an excessive use of antibiotics and has also decreased the fish farmers confidence in the commercially available vaccines. To address this issue two successive collaborative research projects have been established. Initially (MarinVac project), it was anticipated, based on the experience from Norway where furunculosis vaccines efficiently eliminated the disease problem in Atlantic salmon, that the commercially available vaccines could provide sufficient protection when used optimally. Field trials were therefore conducted with the aim of determining the optimal vaccination strategy for the sea reared rainbow trout in DK, particular in terms of time of vaccination before transfer of the fish to seacages. The results revealed that there were equal effects of early and late vaccination (1 year vs 3 month before transfer to sea). And needs for antibiotic treatment against furunculosis occurred in both groups during warm Summer periods. An experimental vaccination trial was then conducted under controlled lab conditions. Two groups of rainbow trout were vaccinated by intraperitoneal (i.p.) injection with two different commercial vaccines, both comprising *Vibrio anguillarum* (*Va*) serotype O1 and O2, and *Aeromonas salmonicida* (*As*) based on cultures of bacteria originally isolated from *Atlantic salmon*. The experiment also included a third group of non-vaccinated controls. All fish were individually chip-tagged. Challenge was performed as a combination of injection- and cohabitation challenge. Six months after vaccination at 10^0C, half of the fish were challenged by ip. injection of *A. salmonicida* bacteria. While the non-vaccinated fish all died within 3 weeks, a certain level of protection was evident among the two vaccinated groups although high mortality also occurred here. No mortality/clinical disease was evident among the noninjected cohabitants at this stage. However, when the water temperature was subsequently raised to 17^0C, the cohabitants started to die. Some variability was evident between replicate tanks, but the overall outcome was that non vaccinated fish performed at least as well as the vaccinated ones. The results demonstrate the importance of the challenge procedure for evaluation of vaccine efficacy under experimental conditions. Although it may be anticipated that the available commercial vaccines can confer some protection against furunculosis in rainbow trout, the results also indicate that there is a need for tailoring the vaccines to the needs of sea reared rainbow trout in Denmark. A new research project (ProFish) has recently been launched in which we will use antigens based on *As* isolates derived from disease outbreaks in sea reared rainbow trout in DK. Also, alternative adjuvants to the traditional mineral oil will be tested, and prime-boost vaccination strategies will be developed.
Both projects received funding from the Danish Council for Strategic Research.

Minutes:
In marine fish farming in Denmark there is problems with outbreaks of furunculosis caused by *Aeromonas salmonicida* during warm summers, despite vaccination. This calls for the need for antibiotic treatment 1-3 times a year.
To address this problem two collaborative research projects has been launched: 1) Improved vaccination strategies in marine aquaculture (MarinVac) (2008-12) and 2) Targeted disease prophylaxis in marine fishfarming (ProFish) (2012-2016)

Selected results were presented, including no difference between early and late vaccination, importance of including cohab challenge in evaluation of challenge and no direct correlation between antibodies and survival.
Optimising of furunculosis vaccines for rearing of trout in DK is needed eg. by tailor-making the furunculosis vaccine and optimizing the antigen and/or adjuvant.

Questions:
Brit Hjeltnes: Which bacteria you used for the challenge?
Niels Lorenzen: The vaccines use commercial strains for the challenge, and we used the one isolated from sea reared rainbow trout in Denmark. We expect some difference but still need to investigate further this aspect.
Perttu Johannes Koski: in Finland the vaccination for broodstock is quite good. I would like to know the situation in Norway.
Brit Hjeltnes: there is an excellent protection against furunculosis and vibriosis, both for salmon and rainbow trout. Environmental condition might influence the appearance of outbreaks
Niels Lorenzen: temperature might have a great influence
Torsten Snogdal Boutrup: One of the problems is that the fish carry furunculosis from fresh water.
Niels Lorenzen: That is true, we need also to improve the quality checking in vaccination procedure (some preliminary data showed that 20% of fish through the automatized vaccine machine was not vaccinated properly)
Sigridur Gudmundsdottir: I suggest to include also fish vaccinated only with adjuvant within the controls.

BREEDING PROGRAM AS A STRATEGY FOR DISEASE CONTROL

Hjeltnes B.[1]

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Abstract:
Breeding program has during many years contributed to the successful development of aquaculture. Important improvements have been achieved in growth performance, maturation and quality. However, limited results have previously been reported on breeding for disease resistance. Recently, use of quantitative trail loci (QTL) has proven to be an important tool for breeding for disease resistance. Promising result has been published on gen-markers for IPN and disease resistance. Norwegian field data suggest there are strong indications that the reduction during the last years in reported cases of IPN is linked to the introduction of QTL-roe. Use of QTL-roe can be an important tool for controlling IPN.

Minutes:
One of the tools available for the control of disease in aquaculture is the implementation of breeding programs for selecting resistant broodstock able to transfer genetic resistance to the eggs.
Different diseases have been targeted in the salmon industry including IPN, ISA, Sea-lice and PD.
So far promising and concrete results have been obtained in IPN resistant salmon strain.
The identification of QTL (quantitative trait loci) and their analysis with experimental infection trials demonstrate that the number of Q allels in parents correlated strongly with IPN resistance in offspring. Resistant IPN Eggs demonstrated 1,3% mortaltiy in the offspring after IPN challenge whereas mortality in offspring from IPN sensitive eggs was 52.5% mortality. The percentage of carriers within the survivors was lower in the IPN resistant fry compared to IPN sensitive. After introduction of QTL-roe the losses due to IPN appear to be very reduced .

Questions:
Niels Lorenzen: do you think there is the need for a new IPN vaccine?
Brit Hjeltnes: The virus is constantly changing so I consider that eradication strategy should involve implementation of effective vaccine and QTL technology.
Niels Lorenzen: Do the QTL fish get infected or they don't suffer of disease?
Brit Hjeltnes: they do get infected but only 10% of survivors carry the virus after the outbreak.
Richard Paley: it seems that 99% of salmon eggs are QTL?
Brit Hjeltnes: These data are provided by QTL companies
Britt-Bang Jensen: Also for CMS the QTL technology seems to provide promising results, the problem is that the QTL for CMS are not in the same loci of QTL for IPN.
Neil Ruane: Is there data available on how the IPN-resistant fish perform when challenged with another disease?
Brit Hjeltnes: I think these data are not available.

SESSION V: Update from the EURL

Minutes : Dr. Niccolò Vendramin and Helle Franck Skall

EURL ACTIVITIES IN 2012

N. J. Olesen, N. Vendramin and N. Nicolajsen
National Veterinary Institute, Technical University of Denmark

Abstract:
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: EHN, EUS; and fish diseases listed as non-exotic diseases: ISA, VHS, IHN and KHV disease.

The 16th Annual Meeting of the NRLs for Fish Diseases was held was held in Aarhus, Denmark, 30-31 May 2012 at the premises of the Section for Fish Diseases at DTU Veterinary. A total of 44 participants from 28 countries attended over the two day period. A report was submitted in August 2011.

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 third year, the proficiency test consisted of two tests, PT1 and PT2. PT1 was designed to primarily assess the ability of participating laboratories to identify VHSV, IHNV and EHNV + SVCV and IPNV (upon request from laboratories being accredited for these pathogens). PT2 was developed in order to test the proficiency of participating laboratories to identify ISA and KHV and in addition also spores of the oomycete *Aphanomyces invadans* causing EUS. Thereby the proficiency test is covering all 6 listed exotic and non-exotic diseases. 44 National Reference Laboratories (NRLs) participated in the proficiency test, the highest number ever. A report was submitted in February 2012. Most laboratories performed very 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 now available at the EURL web page. Diagnostic manual for EUS has been uploaded in 2012. This disease was, however, delisted by the EU Commission in autumn 2012 and might thus be removed from our web page again. We have nevertheless decided to keep it updated untill discussions on listing/no-listing is finalised. Unfortunately the manual on sampling and diagnostic procedures for the listed diseases has still not been adapted by the EU, the diagnostic methods therefore still relies on the former Commission Decision 2001/183/EC for VHS and IHN and 2003/446/EC for ISA while no legislative text exist for KHV, EHN and EUS.

Another important focus area was the development, implementation and validation of diagnostic tools for identification of the listed diseases and their accreditation. One outcome of these efforts was the publication on the generation of a real-time RT-PCR assay for detection of all genotypes of VHSV that has been proposed for the OIE to be used as an alternative to surveillance for VHS by cell cultivation!

During 2012, resources were also used to collate data on surveillance, health categorisation, and diagnostics in EU; to identify and characterise selected virus isolates; to type, store and update a library of listed virus isolates; to develop, update and maintain the database containing information

on fish pathogens (www.fishpathogens.eu); to supply reference materials to NRLs; to provide training courses in laboratory diagnosis; to produce antisera; to update the EURL webpage (*www.eurl-fish.eu*); and finally to attend international meetings and conferences.
The permanent staffs of the Section for Fish Diseases in Aarhus, Denmark consists of approx. 22 academic and technical staff, primarily involved in research, diagnostics and consultancy with special focus on fish virology.

EURL WORKPLAN FOR 2013

Niels Jørgen Olesen, Nicole Nicolajsen, Anemone Olaja, Susie Sommer Mikkelsen, and Niccolò Vendramin

The work plan and the expected outputs from this year is as follows:

	Description	Objectives	Expected outputs
1-1	Annual workshop	Organise and prepare for the 17th Annual Workshop for the National Reference Laboratories for Fish Diseases (NRLs) in 2013	To be held May 2013
1-2	Annual workshop report	Produce a report from the Annual Workshop 2013.	To be finalized and submitted August 2013
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 February 2013 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 at the EURL laboratory facilities. The courses will primarily be for training of staff from NRLs and the content will depend on request from participants.	Provided the necessary cost for conducting training courses will be provided the courses will be held primo February 2013; two courses lasting 3-4-day with expected 15 participants are foreseen.
2-1	Proficiency test	Prepare the Annual Inter-laboratory Proficiency Test year 2013 for the NRLs. The test will include VHSV, IHNV, EHNV, ISAV, KHV and *Aphanomyces Invadans*.	To be shipped October, 2013
2-2	PT report	Collate and analyse information gained from the Inter-laboratory Proficiency Test	Report for the proficiency test 2012 will be submitted February 2013, while results of the 2013 test will be finally collated December 2013
3-1	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 2013
3-2	Antisera	Production of antisera against selected isolates when necessary	Rabbit antisera are foreseen to be produced e.g. against the emerging pathogen nodavirus
3-3	Pathogen library	Update and maintain a library of isolates of Infectious salmon anaemia virus (ISAV), Viral Haemorrhagic Septicaemia virus (VHSV) and Infectious Haematopoietic Necrosis virus (IHNV), Koi Herpes virus (KHV) and enzootic haematopoietic necrosis virus (EHNV) and *Aphanomyces Invadans*.	The library will be updated with approximately 20 pathogen isolates

	Description	Objectives	Expected outputs
3-4	Tissue library	Maintain a library of tissue material from fish infected with listed pathogens	The tissue library will be maintained and updated with new specimens.
4-1	Webpage	Update the webpage for the EURL, www.eurl-fish.eu	Current task making the webpage more interactive and easy accessible.
4-2	Diagnostic manuals	Update the diagnostic manuals for VHS, IHN, ISA, KHV disease, EHN and EUS on the EURL web page.	As the diagnostic manuals were very recently adopted a number of amendments are foreseen to be done. Based on the 16th AM more focus to sampling procedures will be given.
4-3	Fishreflabnet	Establish an interactive network with the NRLs, Fishreflabnet , in order to promote a more proactive data sharing and communication with and between reference laboratories in member states.	An internet based platform for communication and data sharing will be established
4-4	Pathogen characterization	Identify and characterise selected isolates of listed viruses (pathogenicity testing in vivo and in-vitro, serological and genetic characterisation).	The EURL receive every year strains and samples for corroboration of diagnostic results in EU Member states. Regularly these strains must be characterised 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 have proved to be valuable for virus characterisation and molecular epidemiology . The more isolates included the stronger a tool. New databases on other listed and emerging pathogens are in the pipeline. At least 50 new VHS and IHN isolates are envisaged to be included and 1-2 new databases opened (KHV, ISA, SVC, etc.)
4-6	Molecular epidemiology	Perform molecular epidemiology analysis to improve knowledge on diseases spreading mechanisms of viral 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 the listed non-exotic fish diseases.	Real-time PCR have shown to be 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 (KHV, IHN)
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 (e.g. using discontools and similar tools)	It was agreed among the NRL´s in EU that more focus should 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 continued in 2013
5-1	Missions	Organizing missions to relevant laboratories. Missions will	1-2 missions will be conducted, the laboratories to visit have not been

	Description	Objectives	Expected outputs
		focus on NRLs where on-site communication would be beneficial.	appointed yet (await results from the proficiency test 2012)
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.

DRAFT EURL WORKPLAN FOR 2014

Niels Jørgen Olesen, Nicole Nicolajsen, Anemone Olaja, Susie Sommer Mikkelsen, and Niccolò Vendramin

OBJECTIVES FOR THE PERIOD JANUARY - DECEMBER 2014

1. Coordination and training

1-1 Organise and prepare for the 18th Annual Workshop for the National Reference Laboratories for Fish Diseases (NRLs) in 2014.

1-2 Produce a report from the Annual Workshop 2014.

1-3 Collect and report data on the fish disease situation in EU, including all the listed non-exotic fish diseases.

1-4 Facilitate and provide training in laboratory diagnosis. The training courses in methods used for diagnosis of fish diseases is offered annually at the premises of the EURL-Fish. The courses will primarily be for training of staff from NRLs and the content will depend on request from participants. The courses will be conducted autumn 2014

2. Proficiency test

2-1 Prepare the Annual Inter-laboratory Proficiency Test year 2014 for the NRLs. The test will include testing for VHSV, IHNV, EHNV, ISAV, KHV and in addition upon request SVC and IPN. As *Aphanomyces Invadans* causing EUS was delisted autumn 2012 it might upon a decision at the 17th AW be removed from the proficiency test 2014.

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

3. Reagents and products

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

3-2 Production of antisera against selected isolates when necessary

3-3 Update and maintain a library of isolates of ISAV, VHSV, IHN, KHV, EHNV and *Aphanomyces Invadans*.

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

4. Scientific advice and activities

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

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

4-3 Collect information on strain variation occurring within pathogens causing the listed diseases VHS, ISA, EHN and KHV disease and provide recommendations on how to discriminate between various strains.

4-4 Identify and characterise selected isolates of listed viruses (serological and genetic characterisation).

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

4-6 Assessment and standardisation of real-time PCR tests for the diagnosis, identification and typing of the listed non-exotic fish diseases.

4-7 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.

5. Missions

5-1 Organizing missions to relevant laboratories. Missions will focus on NRLs where on-site communication would be beneficial.

5-2 Attending missions, international meetings and conferences in order to be updated on emerging and listed exotic and non-exotic fish diseases.

Apart from all the mandatory plans for next year suggestions for other topics to work on for the EURL would be most appreciated

SUGGESTIONS FROM PARTICIPANTS:

Richard Paley: We might need KHV - Working group. One of the task could be a proper validation of one test for KHV.

Olga Haenen: MALDI-TOF for identification of bacteria could be an interesting topic for a lecture in a big workshop. Proper sampling for fish disease diagnosis, for field workers. Zoonotic bacteria related to aquaculture professionals in the field (field samples, slaughterhouse, risks

Vlasta Jencic: I would like to address non-lethal sampling and serology.

Brit Hjeltnes the OIE has submitted a chapter for determining susceptible species. It will be a topic for commission meeting in October. Need to be discussed.

Niels Jørgen Olesen: EFSA made a report on susceptible species. It seems that there is no proper coordination between OIE and EU. How should we define a susceptible species? In the past there was the list of vector species, then only susceptible species.

Niels Jørgen Olesen: IHN procedure need to be addressed we need to have a validated IHNV real time RT-PCR.

EURL TRAINING COURSE 2013 AND REQUEST FOR IDEAS FOR 2014

Susie Sommer Mikkelsen[1]

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Abstract:
From the 21st to the 31st of January 2013 the EURL offered two training courses. "Sampling and diagnostic procedures for surveillance of listed fish diseases" was a 5-day course focused on the process from inspection of a fish farm to sampling of fish and processing of the diagnostic samples. The course was mainly a hands-on course where the participants were able to get practical experience with some of the most used techniques in fish diagnostics, including cell-culture, ELISA and PCR. The course was followed by 5 participants.
"Advanced biomolecular techniques and bioinformatics" was a 4-day theoretical course focused on PCR, real-time PCR, sequencing and phylogenetic theory. It followed a logical progression from the basic techniques of PCR and real-time PCR, through Sanger-sequencing and next-generation sequencing of the PCR products to phylogenetic analysis and real case-stories interspersed with theoretical assignments. Guest teachers were Dr. Heike Schütze, FLI, Germany and Dr. Marc Engelsma, CVI, The Netherlands. 12 people participated in the course.
I will give you a presentation of the two courses as well as the feedback from the participants. We encourage you to provide topics for next year's courses.

Suggestion for topics on new courses.

Thierry Morin: I would like more technical knowledge for NGS and bio-molecular techniques
Angel Trent: I would suggest a baseline course for food inspector. Clinical signs in the field basic pathology with emphasis of collection of samples, and blood sampling.
Neil Ruane: We would have sent fish inspectors this year, but the problem was that the personnel involved in sampling are not the same as those who perform the tests, so there should be dedicated session for sampling.
Niels Jørgen Olesen: We should keep in mind that our first focus are the laboratory methods. This year we decided to include the farm visit in order to provide a wider understanding of the entire process.
Brit Hjeltnes: I agree that sampling techniques are very important, it could be made with training video they can be distributed easily.
Olga Haenen: Small course on ornamental fish disease?
Niels Jørgen Olesen: we had courses in serology. Could it be a topic of interest? No serological methods are available in our procedures, for instance serology procedures for KHV.

11 labs demonstrated interest in this topic.

RESULTS OF THE PROFICIENCY TEST, PT1 AND PT2, 2012

Niccolò Vendramin, Nicole Nicolajsen, Maj-Britt Christophersen and Niels Jørgen Olesen

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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).
The number of National Reference Laboratories (NRLs) participating in PT1 and PT2 was 43.
The tests were sent from the EURL in the beginning of September 2012.
Both PT1 and PT2 are accredited by DANAK under registration number 515 for proficiency testing according to the quality assurance standard DS/EN ISO/IEC 17043.

PT1 consisted of five coded ampoules (I-V). These ampoules contained IPNV, EHNV, SVCV, IHNV and VHSV, respectively, see table 1. The proficiency test was designed to primarily assess the ability of participating laboratories to identify the listed fish viruses VHSV, IHNV and ENHV (Council Directive 2006/88/EC) and the non-listed viruses SVCV and IPNV if present in the ampoules, bearing in mind that the test ampoules also could contain other viruses (e.g. other fish rhabdoviruses, ranaviruses, or birnaviruses). In addition the participants were asked to quantify the viruses in PT1 by titration in order to assess the susceptibility of their fish cell lines for virus infection. Participants were encouraged to use their normal standard laboratory procedures. However, the identification should be performed according to the procedures laid down in Commission Decision 2001/183/EC using fish cell cultures followed by e.g. ELISA, PCR, immunofluorescence (IFAT) or neutralisation test.
If ranavirus was present in any of the ampoules, it was mandatory to perform sequence analysis or a restriction endonuclease analysis (REA) of the isolate in order to determine if the isolate was EHNV or another ranavirus and it was recommended to follow the procedures described in Chapter 2.3.1 in the OIE Manual of Diagnostic Tests for Aquatic Animals 2009. Laboratories were encouraged to identify VHSV and IHNV isolates as far as possible by means of genotyping in addition to the standard methods. It was recommended to use the genotype notification described in Einer-Jensen et *al*. (2004) for VHSV and in Kurath et *al*. (2003) for IHNV. Laboratories were encouraged to submit all sequencing results used for genotyping the isolates.

PT1 Conclusion

The inter-laboratory proficiency test 2012 was conducted without major constraints. 92% of parcels were delivered by the shipping companies within 8 days after submission. It was, however, unfortunate that two parcels were 20 days on the way and one parcel was 43 days on the way before delivered to the laboratory primarily due to border controls. Two parcels never left the EURL. In one case this was dued to delivery restriction for such reagents (Iran), in the other case because the fetal bovine serum (FBS) used was from a country not certified free from foot and mouth disease.
In the meantime the batch of serum currently used in the EURL for cell culture has tested negative for foot and mouth disease virus (FMDV) following accredited procedures.
In 2009 EHNV was included in the proficiency test for the first time this year 36 participants were able to correctly identify the virus. Of the laboratories performing PCR based methods, 33 laboratories performed sequencing. Of these laboratories all correctly identified the content. Two laboratories performed REA and one laboratory performed restriction enzyme fragmentation.

PT2
It consisted of four coded ampoules (VI-IX). The ampoules contained ISAV and KHV. Furthermore, one ampoule contained *Aphanomyces invadans* and one sterile pyrogen free water. The test was designed to primarily assess the ability of participating laboratories to identify the notifiable fish pathogens ISAV, KHV and *A. invadans* (listed in Council Directive 2006/88/EC) if present in the ampoules, bearing in mind that the test ampoules could also contain other pathogens. Participants were expected to use their normal PCR or real-time PCR methods for detection of the pathogens. It was not mandatory to grow KHV and ISAV on cell cultures in this test, but the viruses were not inactivated and, thus, it might had been possible to replicate them in cell cultures.
If present, only **inactivated** *A. invadans* was included in the ampoules.

PT2 conclusion
Considering that this was the third time that the EURL provided a proficiency test on ISAV and KHV identification, and the second time that the EURL provided a proficiency test on *A. invadans*, we consider that most participants obtained satisfying results. Out of 34 laboratories testing for *A. invadans* all 34 identified the pathogen in ampoule VI. Out of 38 laboratories performing KHV identification, 36 laboratories identified KHV in ampoule VII. Out of 39 laboratories 32 laboratories identified Not *A.invadans, KHV or* ISAV in ampoule VIII. Out of 38 laboratories performing ISAV identification 36 identified ISAV in ampoule IX. Very significant improvement in the proficiency of identifying and typing these pathogens has been observed during these 3 years. In autumn 2012 the European Commission decided to de-list EUS and it is officially no more considered as an exotic disease in the Union. However we find that a certain level of preparedness for the introduction of this disease in European aquaculture should be maintained. But it is still unclear whether the pathogen will be included in future inter-laboratory proficiency tests or not and the topic will be discussed at our next Annual Meeting in May 2013.
It is an appreciated matter of fact that many laboratories are putting efforts in performing genetic characterizing 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.

The results of the proficiency tests will be further discussed at this presentation.

Minutes:
Every participant has received a dedicated report and a general report is available on the internet. More than 95% obtained maximum score this year.
Procedures on how the Proficiency test is prepared are described to participants in order to make them acknowledged of the entire process including "customer" satisfaction questionnaire.
The possibility to include an extra PT (PT3) with non-listed but relevant diseases will soon be evaluated
It will be evaluated soon the possibility to include an extra PT (PT3) with non-listed but relevant diseases.

Questions:
Irene Øperveit: Will you charge us for the shipment of PT3?
Niels Jørgen Olesen: for the European NRLs we do not charge you, as this is put into our budget. But if we start to provide PTs for non-listed diseases we may have to charge you as we do not have a budget for this.
Niels Jørgen Olesen: Who will like us to keep EUS in the ringtest for 2014.

9 laboratories want to keep EUS in the PT, 9 want it to go out. SVCV and IPNV are included in the test already. There were requests for including SAV in the tests. If you have other requests please contact us. With EUS, it is still a listed disease for the OIE.
Neil Ruane: I think many laboratories would be happy to participate in a PT3 with non-listed diseases and it would be nice if you could send out a questionnaire on what pathogens people would like to have included.
Niels Jørgen Olesen: OK we should just bear in mind that there already are commercial PTs which we should not compete with.

GREETINGS AND CONCLUSION OF THE MEETING

The next meeting will be held at the end of May 2014. It will probably be organized in our new facilities here in Copenhagen, we could also consider other facilities in Europe but we have to provide proper justification to the commission to do so. Of course scientifically speaking it would be very interesting to visit other laboratories.

Thanks a lot to the people arranging the meeting as well as those of you who helped running the meeting by being chair persons, presenting stuff and being here.