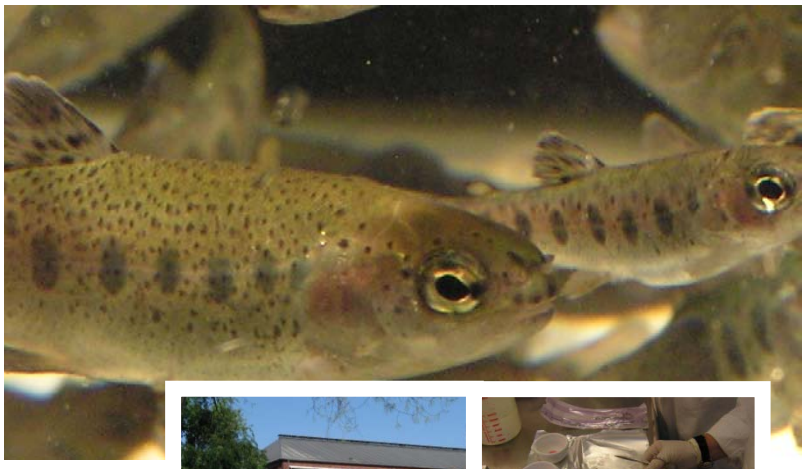
European Union Reference Laboratory for Fish Diseases

National Veterinary Institute, Technical University of Denmark, Aarhus



15th Annual Meeting of the National Reference Laboratories for Fish Diseases

Aarhus, Denmark May 26-27, 2011

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

Indhold

Introduction and short summary

26-27 May 2011 the 15th annual meeting of the National Reference Laboratories for fish diseases was held in Aarhus, Denmark, at a rented nearby facility of Aarhus University. A total of 52 participants from 33 countries attended over the two day period. There were four sessions with a total of 33 presentations, 9 of which were given by invited speakers.

The scientific programme of the Annual Meeting was diverse and covered many topics of current interest. The meeting was opened with the traditional session on update of fish diseases in Europe, where once again 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 2010 were provided on the basis of the results from the survey and diagnosis questionnaire. Results of a questionnaire send to fish-pathology experts in the Mediterranean area were the basis of a following talk providing an update on fish disease situation in Mediterranean aquaculture. Subsequently, needs and requirements for rapid and efficient responding to emerging diseases were provided followed by an update on the disease situation in Norway. This was followed by a talk on IPNV distribution in Austrian aquaculture 1993-2010 and a talk on the VHSV eradication program in Denmark. Afterwards, three talks on molecular epidemiology from Denmark, Poland and Germany, respectively were presented. The session was finished with a talk on *vibrio* infections in Dutch aquaculture.

The session on technical issues related to sampling and diagnosis were started by a presentation on the new EU manuals on sampling and diagnostic procedures and the role of the www.eurl-fish.eu web page. Next were three talks on diagnostic methods; one for identification of EHNV and two for identification of *Aphanomyces Invadans*; followed by a plenum discussion on options for surveying for EUS in EU in farmed and ornamental fish. After a short coffee break a talk on health categorisation of fish farms in Europe 2010, based on collected answers of the questionnaire on surveillance and diagnostic, was presented. Then a talk high lightening some of the challenges concerning the implementation of the new legislation on aquatic animal health surveillance in Europe, was provided. The session was ended with three presentations, one on a new real time LAMP assay for KHV detection, one on the origin of diagnostic fish cells and at last one on challenges in development of real-time PCR diagnostic assays.

In the evening a banquet dinner was held at Restaurant “Koch”, located at the harbour side, downtown Aarhus.

The last day was opened by an update session on scientific research. The first part of this session was focussed on ISA. Initially, a chronicle of events relating to a small scale ISA epidemic situation was described for a specific Norwegian area containing several salmon farms. Subsequently, an overview was provided on ISA and HPR0 strains, followed by presentation of a study on HPR0 appearance in fresh water at the Faroe Island. The last talk on ISA was on HPR0 detection in Denmark in combination with a comparison of the criteria for diagnosis of ISA as described by EU and by OIE. The ISA topic was ended by a discussion in plenum on how the criteria for diagnosis of ISA should be in relation to an identification of HPR0. At the end of this session a talk on a PD-vaccine that efficiently reduces severity of disease outbreak in Norwegian aquaculture was presented. This was followed by a talk on an Epizone extension project on “Management, control and surveillance of VER in aquaculture” and finally a talk on serological tests for specific antibody detection against VERv ended the session.

The annual meeting ended with the traditional update from the EURL. The results of the two proficiency tests, PT1 and PT2, 2010 were presented. Furthermore, the annual training course provided by the EURL was announced and topics were discussed. A report of EURL activities from year 2011 was given. Finally, proposals on the EURL work plans for 2012 were discussed.

Minutes from the meeting were taken by Helle Frank Skall, Søren Peter Jonstrup and Torsten Boutrup, and have afterwards been sent to presenters for correcting in order to avoid misunderstandings. The minutes are included in this report together with abstract and comments from the discussions. Nicole Nicolajsen 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 Nicole Nicolajsen, Søren Kahns, and Niels Jørgen Olesen, with the help from the rest of the fish disease section at DTU Vet.

The meeting next year is tentatively planned to be in week 21, most likely from 23-25 May 2012 but more details will follow.

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

Århus, 07 September 2011

Niels Jørgen Olesen and Søren Kahns

Programme

Thursday May 26th
Annual Meeting of the National Reference Laboratories

8:45 – 9:15	**Registration and welcome address**
9:15 – 09:30	*Welcome Address and announcements - Søren Kahns and Niels Jørgen Olesen*

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

Chair – *Olga Haenen/Brit Hjeltnes*

9:30 – 9:50	Overview of disease situation in Europe - *Niels Jørgen Olesen*
9:50 – 10:10	Update on fish disease situation in the Mediterranean area – *Giuseppe Bovo*
10:10 – 10:35	Emerging diseases – an overview – *Stephen Feist*
10:35 – 10:55	Update on the Fish Disease Situation in Norway– Brit Hjelt*nes*
10:55 -11:10	Distribution of IPNV in Austria 1993-2010 - *Oskar Schachner*
11:10 - 11:35	Coffee break
11:35 -11:50	Surveillance and eradication of VHS in Denmark – *Stig Mellergaard*
11:50 - 12:15	Danish genotype Ia VHS viruses constitute a subgroup distinct from isolates causing outbreaks in other European countries – *Søren Kahns*
12:15 – 12:30	Characterisation of Polish VHSV isolates (2005-9) – *Marek Matras*
12:30 – 12:45	Characterisation of German IHNV and VHSV isolates – *Heike Schutze*
12:45 – 12:55	Vibrio infections in Dutch fish culture – *Olga Haenen*
12:55 – 13:45	Lunch

SESSION II: Technical issues related to sampling and diagnosis

Chair – *Stephen Feist/Richard Paley*

13:45 - 14:05	The new EU manuals on sampling and diagnostic procedures and the role of the www.EURL-fish.eu web page - *Niels Jørgen Olesen*
14:05 - 14:25	Diagnostic methods for identification of EHNV and other ranaviruses – *Heike Schuetze*
14:25 - 14:55	Epizootic ulcerative syndrome (EUS): Development and implementation of diagnostic methods – *Olga Haenen*
14:55 – 15:10	Sporulation of *A. Invadans* - *Christian Fry*
15:10 – 15:20	Options for survey for presence of *A. Invadans* in EU in farmed and ornamental fish – discussion in plenum – *Niels Jørgen Olesen*
15:20 - 15.50	*Coffee break*
15:50 – 16:10	Health categorisation of fish farms in Europe – *Niels Jørgen Olesen*
16:10 – 16:30	Challenges regarding implementation of the new legislation on Aquatic Animal Health Surveillance in Europe – *Britt Bang Jensen*
16:30 – 16:45	Development and assessment of a real time LAMP assay for KHV– Richard Paley
16:45 – 17:00	Fish cells – some remarks to induce discussion – *Heike Schutze*
17:00 – 17:15	Pitfalls and challenges in development of real-time PCR diagnostic assays – *Søren Peter Jonstrup*
18:30 – 21:30	***BANQUET DINNER at Koch'erier***

Friday 27 May
Annual Meeting of the National Reference Laboratories Continued

SESSION III Scientific research update

Chair – *Søren Kahns*

9:00 – 9:30	Infectious salmon anaemia (ISA) in Norwegian salmon farming, a chronicle of events relating to a small scale epidemic in the Southern and middle parts of Troms county 2007- 2010 – *Einar Karlsen*
9:30 – 9:50	Infectious salmon anaemia and HPR0 strain – an overview – *Eann Munro*
9:50 – 10:10	Detection of low-pathogenic Infectious Salmon Anemia Virus (ISAV-hpr0) in freshwater in the Faroe Islands – *Debes Christiansen*
10:00 – 10:20	Detection of HPR0 in Denmark and Criteria for diagnosis of ISA– *Helle Frank Skall*
10:20 – 10:30	PD-vaccine is effective in reducing severity of disease outbreaks in Norwegian aquaculture – B*ritt Bang Jensen*
10:30 – 10:50	Extension of Epizone – Management, control and surveillance of VNN in aquaculture - *Giuseppe Bovo*
10:50 – 11:05	Serological tests for specific antibody detection in European Sea bass (*D. labrax*) against VERv. - *Niccolo Vendramin*
11:05 – 11:30	Coffee break

SESSION IV Update from the EURL

Chair – *Niels Jørgen Olesen*

11:30 – 11:45	EURL activities in 2010 - *Niels Jørgen Olesen*
11:45 – 12:00	EURL workplan for 2010; Ideas and plans for 2011 - *Niels Jørgen Olesen*
12:00 – 12:05	EURL training course 2011 and Request for ideas for 2012 – *Søren Peter Jonstrup*
12:05 – 12:25	Results of the proficiency test, PT1, 2010 – *Søren Kahns*
12:25 – 12:40	Results of the proficiency test, PT2, 2010 – *Søren Kahns*
12:40 – 13:00	Next meeting and end of 15th Annual Meeting - *Niels Jørgen Olesen*
13.00	***Sandwiches and goodbyes***

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

Chair*: Olga Haenen/ Brit Hjeltnes*
Minutes: *Torsten Boutrup/ Søren Peter Jonstrup*

OVERVIEW OF THE DISEASE SITUATION AND SURVEILLANCE IN EUROPE IN 2010

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 2010 it comprise 3 parts:

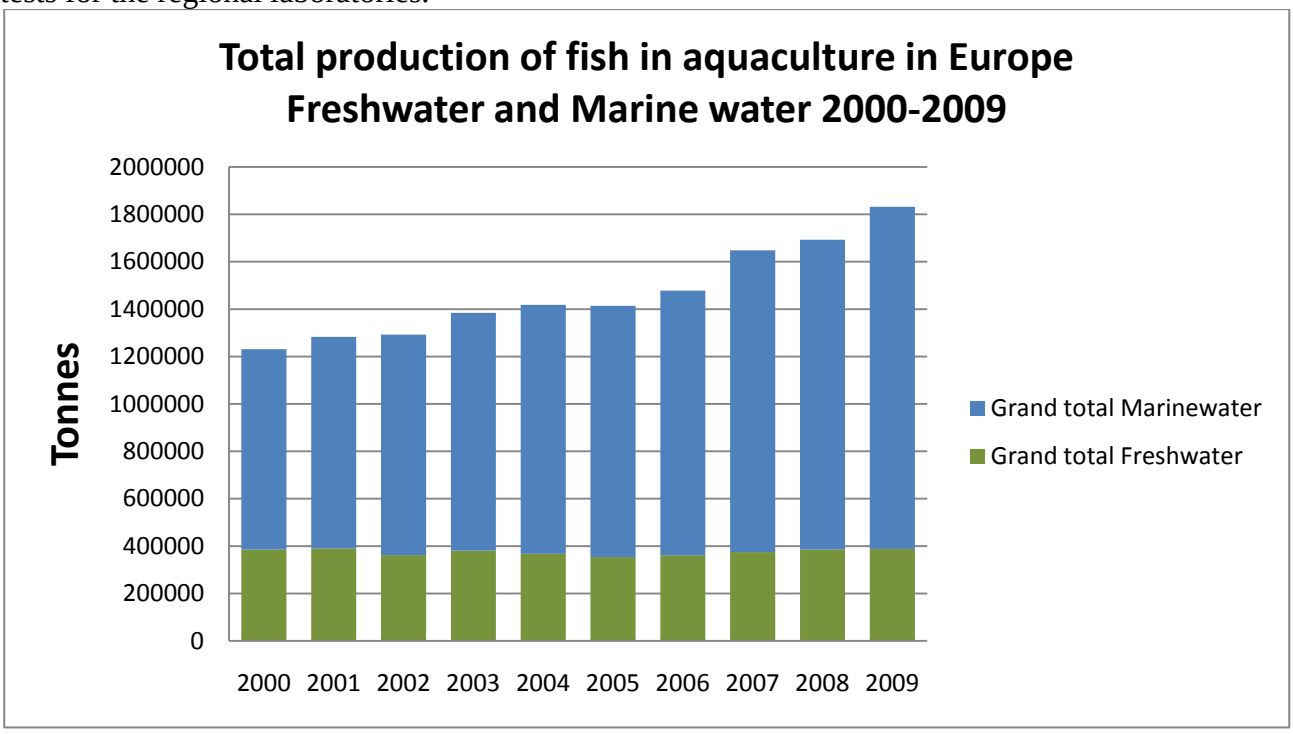
1. General data on production type and size, health categorization of fish farms according to Council Directive 2006/88/EC, and information on national surveillance programmes.
2. Epidemiological data on the disease situation in each Member State with focus on the listed diseases but also including other 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. Unfortunately this database does not include information on the number and size of fish farms, which are epidemiologically important data. The production in 2009 is almost the same as in 2008 and has risen after a decrease from 2003-2006. Data from 2010 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 is mainly at the Atlantic Sea coast line of the Northern European countries. Rainbow trout are produced in most European countries, whereas carp production is mainly in the Eastern part of Continental Europe. The production of sea bream, sea bass and tuna have increased significantly in the Mediterranean countries. The production in Turkey raised from 66.000 to 76 000 t last year and Turkey has become one of the countries included with the highest production. Among other fish species of interest are pike-perch (408t), eel (6391t), sturgeon (3789t), cod (22729t), turbot (9238t), and halibut (1806t).

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 and IHN in many countries. For VHS the infection status in only known for 36% of the farms, for IHN the situation is known in 38% of the farms. While for KHV the disease situation is unknown on 94% of the farms! For farms producing Atlantic salmon, the infection status for ISA is known for nearly all farms. ISA is still a problem in Norway. The finding of ISAvirus HPR0 poses some problems regarding the health categorisation of salmon farms.

Many countries have surveillance programmes for SVC (20 of 29 countries), BKD (16 of 32 countries), IPN (22 of 32 countries) and *Gyrodactylus salaries* (8 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.
There are very large differences between countries on how many samples are tested on cell cultures, ranging from < 100 to several thousands. PCR is coming up in many countries, and the large number of PCR-tests conducted in some countries mostly reflects the KHV and ISA testing.
About a third of the countries have regional laboratories, and of these countries, 8 of 11 organize ring tests for the regional laboratories.



Minutes:

The size of production in Europe seems to be stable with a slight increase especially for saltwater species such as cod, turbot and halibut. Within the EU, central plans are to increase aquaculture production. Data can be somewhat misleading especially on farm size since no data has been reported for France and Hungary. Especially for KHV and ISA many farms have been put in category III, for ISA this is to a large extent due to Norway has decided to categorise all farms as III and this also is the case for VHS in Norwegian saltwater facilities rearing rainbow trout. For other diseases than VHS, IHN, KHV, SVC and ISA main problems in northern part of Europe is IPN, HSMI, CMS, PD and ERM. In mid/central Europe ERM, RTFS and furunculosis seems to be the most important diseases, and Noda virus is a pathogen of great importance in Southern Europe/Mediterranean area. For the listed diseases there seems to be a significant underreporting especially on VHS.

Questions:

No questions.

UPDATE ON FISH DISEASE SITUATION IN THE MEDITERRANEAN AREA

Bovo G.
Istituto Zooprofilattico Sperimentale delle Venezie
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Abstract:
Despite several new species have been introduced, since several years, in Mediterranean aquaculture, in order to diversify the market, European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) are still the most widely farmed species. The production of these two species represents more than 95% of the total production while the remaining is obtained by different promising candidates species, including Senegalensis sole (*Solea senegalensis*), meagre (*Argyrosomus regius*) and northern blue fin tuna (*Thunnus thynnus*), for which some hatcheries have recently started experimental reproduction. The farming of sea bass and sea bream shows a continuous upward trend despite the presence of several important diseases. Viral encephalopathy and retinopathy (VER) still plays a key role in some areas where, in critical situations, mortalities ranging from 30-40% are usually observed in sea bass cages. Moreover in recent years serious epizootics, affecting sea bream larvae, previously considered a resistant species, have been reported.
Marine Flexibacteriosis, caused by *Tenacibaculum maritimum*; Vibriosis, caused by *Listonella anguillarum* and Pasteurellosis caused by *Photobacterium Damsela subsp. piscicida* are considered the major bacterial diseases.
Gill flukes (*Diplectanum aequans and Sparicotyle chrysophrii*); gill crustacea (*Ceratotoa oestroides*) and traditional "old" protozoans (*Cryptocarion irritans and Amyloodinium ocellatum*) can cause high mortalities particularly in ponds, if not properly and promptly treated.
Among the issues still unresolved Winter Syndrome, affects mainly 1-year-old sea bream causing mortalities ranging from 5-15%. The therapeutic treatment addresses great attention to the diet, especially at the end of Summer and the approaching winter season.
Trout farming which is a well developed industry in some regions bordering the Mediterranean may be affected by several biological risks.
Among viral diseases viral haemorrhagic septicaemia (VHS) is certainly the most important problem causing serious losses in those areas characterized by low water temperature.
Rainbow trout fry syndrome (RTFS) is responsible for significant mortalities in salmonids, during juvenile stages, particularly if not treated promptly. Lactococcosis, due to *Lactococcus garvieae* considered in past years an emerging or re-emerging problem, seems to be now, since a couple of years, in a regressive phase, possibly due to the greater availability of water.
Rainbow trout gastroenteritis syndrome (RTGE), is considered, in some regions, an emerging problem. Mortality is generally low (< 5%) but feed intake and growth performances are significantly affected. The diagnosis is based mostly on clinical signs and detection of the Gram + *Candidatus arthromitus* which has been tentatively associated to the disease.

Minutes:

Compared to previous questionnaires Tunesia has been attributed with an aquaculture production. Marbled trout even though not listed as a susceptible to VHSV is so. In the Mediterranean area 100.000 tons of salmonid species and 300.000 tons of marine species are produced and the presence of certain diseases differs upon geography e.g. mycobacteriosis is considered a problem in Israel. In general some old diseases still affects the production with some modifications, for instance RTFS

seems to affect larger fish than fingerlings and lactococcosis seems to be in regress these years and Noda virus infection has been seen to be able to have serious affect on sea bream larvae. New or unresolved problems include abnormal carp mortality and winter syndrome, the latter might be a combination of several factors, however, *Pseudomonas anguilliseptica* is often isolated in connection with the disease. Furthermore, rainbow trout gastro enteritis RTGE seems to be of increased importance in some regions. An increasingly important aquaculture species is the northern blue fin tuna. Due to the novelty of this production and the management of this production new disease situations might be seen in coming years.

Questions:

Olga Haenen: About the abnormal carp mortality, have you looked for picorna virus?

Giuseppe Bovo: This has not been detected.

Athanasios Prapas: You say that mortality due to Noda virus as high as 20 % can be seen in sea cages with larger specimens. Is that right? I have only seen this in fry and smaller specimens.

Giuseppe Bovo: Yes there are several reports on high mortality in larger fish due to Noda virus.

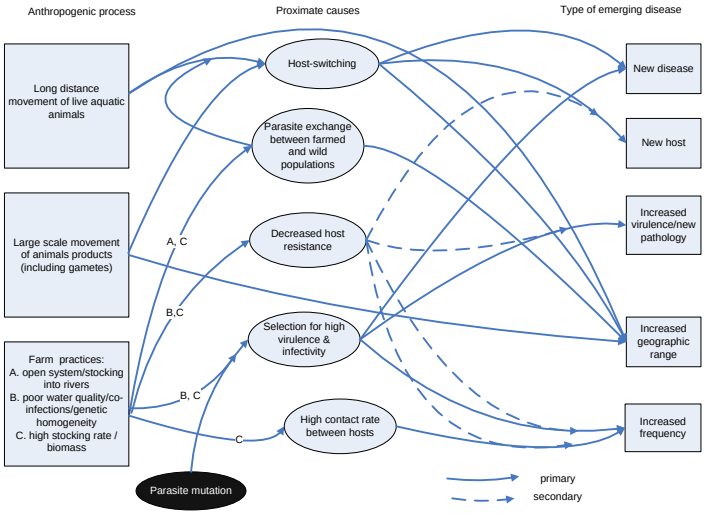
EMERGING DISEASES – AN OVERVIEW

S. W Feist, M Thrush, P Dunn & E Peeler
Cefas Weymouth Laboratory, Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB. UK

Abstract:
An emerging disease can be defined as 'A newly recognised *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 unrecognised pathogenic agent or a *disease* diagnosed for the first time and which has a significant impact on aquatic animal or public health' (OIE Aquatic Animal Health Code, 2010). There is a requirement of EC 2006/88 (report & control), OIE (notify) and the new EC Animal Health Regulations for addressing the detection of new diseases. This presentation highlights the regular occurrence of new and emerging diseases in aquaculture, the possible drivers for their emergence such as translocation of non-native species and the processes underpinning disease emergence (e.g. host switching, high contact rate between hosts, pathogen mutation, selection for high virulence and infectivity, lowered host resistance and parasite exchange between farmed and wild populations. An approach for the analysis of global information using electronic sources is described together with an analysis of trends in emerging diseases for the previous eight years. Key messages are that:

Emerging diseases are a natural component of ecosystems, they continue to be a significant threat to aquatic systems
Need for vigilance
Critical need to experienced diagnosticians, especially histopathologists
Need to develop a robust case definition and establish aetiology as soon as possible
Need to understand the drivers for disease emergence
Multidisciplinary approach essential
Rapid response required under EC 2006/88 (report & control) and the new EC Animal Health Regulations

Hypothesised relationships between anthropogenic processes and types of disease emergence in freshwater aquatic animals



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

Minutes:

To address problem associated with emergence of new diseases, it is of importance to understand the drivers for the appearance of increased potential in an aetiological agent to produce disease, and to acknowledge that this is a naturally occurring process which continually must be taken into consideration to be present and be addressed by fast and rational reporting and swift diagnostic work. Further diagnostic works needs to be multidisciplinary; in this sense histopathology is of importance to establish case definitions on which aetiology, epidemiology and molecular data can be coupled.

Questions:

Stig Mellergaard: The movement of live aquatic animals, especially ornamental fish and molluscs seems to be one of the most serious routes of introducing new disease to an area.

Stephen Feist: I agree about this.

Britt Hjeltnes: Are you able to recall any emerging disease situation that has been handled efficiently?

Stephen Feist: I think that the UK handling of the VHS outbreak was dealt with in the correct manner.

Ole Bendik Dale: Interesting cases where histopathology shows a lesion pattern that indicate a new disease situation often is missed because upon contacting the farmer it turns out that the fish has been destroyed. In some situation where it could be of importance to catch emerging disease situation might be missed due to lack in communication.

UPDATE ON THE FISH DISEASE SITUATION IN NORWAY

Brit Hjeltnes,
Norwegian Veterinary Laboratory

Abstract:
In 2010, 944 000 tons (harvest statistics) of Atlantic salmon, along with an estimated 55 700 tons of rainbow trout, 19 700 tons of cod, 1800 tons of halibut and 1500 tons of other species e.g. coalfish, Arctic char and halibut, were produced (Kontali Analyse AS). Production related losses remain significant, and a large proportion of these losses are related to disease and production conditions.
Pancreas Disease (PD) has been a dominant disease in salmon farming in recent years. Both the industry and the authorities have worked hard towards control of this disease. During 2010 a "min-epidemic" was experienced in the Ry Region, resulting in an increase in the total number of outbreaks from the previous year. Despite this, losses to PD were generally lower. Further spread of the disease northwards was avoided and it appears that the disease has been eradicated from an area in Northern Norway which had experienced repeated outbreaks in recent years.
The number of infectious salmon anaemia (ISA) outbreaks continued to decrease in 2010, although a core area for this disease still exists in Northern Norway.
Infectious Pancreatic Necrosis (IPN) is no longer a notifiable disease, but the losses associated with this disease were again high in 2010, with aggressive outbreaks in some regions. For this disease and the "new" viral diseases like heart and skeletal muscle inflammation (HSMI) and cardiomyopathy syndrome (CMS), the possibility of coordinated eradication strategies should be considered.
AS in 2009, the major fish health challenge during 2010 was again salmon lice infestation. Problems of reduced sensitivity and development of resistance to treatment have increased and as a consequence large numbers of lice were recorded throughout the autumn. Successful salmon louse treatment is dependent on coordinated control strategies requiring extensive cooperation throughout the Norwegian aquaculture industry. While new medications are necessary, they will only provide a breathing space for establishment of other sustainable control strategies. Increased use of wrasse will constitute an important element in such strategies, but will also increase the possibility of other types of disease and disease transmission. Although a vaccine should be an important element in salmon louse control in the longer term, there remains much uncertainty related to both developmental time and effect.
A public surveillance system is under development, and its efficiency will greatly depend on reports of possible resistance development and treatment failure by the fish health services and the industry in general.

Minutes:

This overview is from the yearly report on the disease situation on salmonid species and other marine species in Norway. The report can be found on the Norwegian Veterinary Institutes homepage. HSMI is widespread constituting low to moderate mortality; the aetiological agent piscine reovirus is commonly detected also in healthy fish. CMS is seen in large fish with mortality rates commonly up to 20 %, in 2010 some cases with high mortality were recorded. In conjunction with CMS the new toti virus, piscine myocarditis virus; is found only in diseased fish. The high impact of IPN virus is seen especially in larger fish, the general absence of disease in fry and fingerlings might be due to the possibility for better management procedures in hatching facilities e.g. manipulation with water temperature. Increased gill problems are seen associated with microsporidian parasites. In 2010 two new findings of *Gyrodactylus salaris* were recorded in wild salmon populations. Major health issues in cod are *Fransicella, Vibrio* and atypical *Aeromonas salmonicida* infections. In halibut atypical

Aeromonas salmonicida infections are a problem. Wrasse is used as cleaner fish in the battle against sea lice, 30 million of these is expected to be used yearly in near future disease problems that has been reported in these fish is *Vibrio* infections and infections with atypical *Aeromonas salmonicida.*

Questions:

Eann Munro: What is done with the wrasse after use, do they die, are they released or re-used?

Brit Hjeltnes: No definitive strategy. Many will die in cages ant the rest are often released, re-used. Management strategies are getting higher priority as the number of wrasse used is increasing.

Stephen Feist: Your data on the cod production seems to be in opposition to Niels Jørgens presentation where there was a slight increase in cod production and expectation of higher production. Is this due to *Fransicella* infections?

Brit Hjeltnes: The Norwegian production has stabilised at a lower level, this is both due to *Fransicella* and to low market prices.

Richard Paley: For IPN there has been significant progress in selective breeding for resistance – have you seen any had any visible impact of this on the incidences of disease outbreaks?

Brit Hjeltnes: There are eggs available claimed to have increased resistance. However this has not been confirmed by epidemiological data.

DISTRIBUTION OF IPNV IN AUSTRIA 1993-2010

Oskar Schachner, Andrea Dressler, Hatem Soliman, Mansour El-Matbouli
Fishmedicine and Livestock Management
Dept. for Farm Animals and Vet. Public Health
University of Veterinary Medicine
Vienna, Austria

Abstract:

According to the laboratory data covering a period of 18 years IPNV is spread over the whole area of Austria at moderate density. The annual proportion of IPNV-infected facilities never exceeded 16%. By means of 2-4 fish cell lines the virus has been isolated either from visceral organs or gonadal products of mainly clinically healthy fish belonging to 6 salmonid species as well as from grayling and 2 cyprinid species. Most of the isolates exhibited equal infectivity in the different cell lines. Only some of them produced different titers in epitheloid and fibroblastoid cell morphotypes. CHSE-214 cells revealed to be most susceptible to the whole collection of aquabirnaviruses.
A phylogenetic analysis of VP2 gene sequences extending over a 1180 bp segment revealed marked diversity between some of the isolates, which could not be related to different host species or cell culture preferences.

Minutes:

When performing VHSV and IHNV surveillance on cell culture in Austria since more than 18 years all samples from salmon from fish eliciting a CPE are tested for IPNV as well. This practice provides a general insight into the prevalence of IPNV in Austria. IPNV was isolated in 2-4 cell lines and identified using TEST-LINE ELISA and/or BIOX IFAT. According to host species and the proportion of facilities tested positive for IPNV brook trout seemed to be the most susceptible species, rainbow trout intermediate and brown trout the least susceptible. Most of the isolates grew equally well on BF-2, RTG-2, EPC and CHSE cells. Some of them displayed preferences for a distinct cell line. Generally CHSE cells seemed to be most susceptible to the whole range of aquabirnaviruses. Even though CPE appeared sometimes later, titres became highest in CHSE cells. Independent of the fish host species and the cell line used for isolation, phylogenetic analyses revealed significant differences between isolates. The isolates fell in two VP2 groups with as little as 20% identity between groups for some isolates. Some isolates fell into a non-typable group.

Questions:

Guiseppe Bovo: Are there any information on the presence of IPNV in hatcheries?

Oskar Schachner: There is only limited information.

SURVEILLANCE AND ERADICATION OF VHS IN DENMARK

Stig Mellergaard
Danish Veterinary and Food Administration, Division on Animal Health
Denmark

Abstract:

Denmark was granted co-financing from the European Fisheries Fund for an eradication programme for VHS in 2008. The programme was initiated in spring 2009. Apart from a single VHS outbreak in February 2009, just before the start of the eradication programme, no new outbreaks have been observed since then. Hence, Denmark has been free of VHS for more than two years.
A summary of the basic principles in the eradication programme will be presented.

Minutes:

254 Danish fresh water fish farms are category I for VHSV. The marine farms (28) are put in category III to prevent VHSV spreading from marine wildlife to inland farms. 61 inland farms are in category II and are undergoing an eradication program to obtain category I status. Final VHS-eradication program runs from 2009-2013 and is funded by the European Fisheries Fund after national priority. Initially, risk farms were fallowed and wild rainbow trout in risk area caught by electro-fishery. If a new VHS outbreak should occur, a mandatory and immediate stamping-out with compensation will be carried out on the infected farms/sites. So far the project has been very successful and spending has been far below budget.

Questions:

No questions.

DANISH GENOTYPE IA VHS VIRUSES CONSTITUTE A SUBGROUP DISTINCT FROM ISOLATES CAUSING OUTBREAKS IN OTHER EUROPEAN COUNTRIES

S. Kahns[1*], H.F. Skall[1], R.S. Kaas[2], B. Bang Jensen[3], S.P. Jonstrup[1], D. Stone[4], N.J. Olesen[1]
[1] *National Veterinary Institute, Technical University of Denmark, Aarhus, Denmark*
[2] *National Food Institute, Technical University of Denmark, Kgs Lyngby, Denmark*
[3] *Norwegian Veterinary Institute, Oslo, Norway*
[4] *CEFAS Weymouth Laboratory, Weymouth, Dorset, UK-England*

Abstract:

Viral haemorrhagic septicaemia (VHS) is causing significant economical losses in European rainbow trout production. The disease is caused by a rhabdovirus (VHSV). The virus can be divided into four genotypes with additional subgenotypes of which the main source of outbreaks in European rainbow trout farming is caused by genotype Ia isolates. Danish aquaculture has been considered endemic infected with VHSV since the first outbreak of the disease was observed in the 1950's. The number of infected rainbow trout farms was at its highest level displaying ~ 400 infected farms in the mid 1960's, after when the number of infected farms have been significantly reduced. A, hopefully, final eradication program, approved by the European Union, was initiated April 1, 2009 with no outbreaks since then. If the eradication plan is successful, Denmark will be regarded as EU category 1 (officially declared free of VHS) on April 1, 2013.
In order to characterize the population of VHSV in Danish aquaculture we collected strains causing outbreaks in the period 2004 – 2009. Phylogenetic analyses using full length G-gene sequences revealed that all collected isolates belong to the group of genotype Ia isolates. Interestingly, the phylogenetic analyses revealed that the group of genotype Ia isolates can be divided into two subgroups. The group that we designate Ia-1 consists mainly of Danish isolates and seems to have evolved in the endemic infected Danish water systems. Furthermore, the Ia-1 subgroup apparently consists of several sub-clades of which some have been eradicated whereas others have developed into those that were found up until 2009. The other subgroup designated Ia-2, consists mainly of isolates causing outbreaks in rainbow trout farms in continental European countries other than Denmark.
There are a few non-Danish isolates present in the Ia-1 group, as well as there are a few Danish isolates present in the Ia-2 group. This study presents how molecular tracing can be used to elucidate virus transmission pathways between international as well as regional fish farms, and will be important in future work aiming at preventing future spreading of fish pathogens.

Minutes:

All sampled Danish VHSV isolates from 2004-9 were sequenced. All isolates sampled from rainbow trout were characterized as genotype Ia isolates. Phylogenetic analyses including isolates from all over Europe showed that the most of the Danish isolates clusters into a defined subgroup. Some of the Danish subclades of viruses are eradicated from the environment. However, the analyses show that infective VHSV might survive for up to four years between causing outbreaks.

Questions:

Guiseppe Bovo: Did you see any influence related to fish species?

Søren Kahns: We have mainly looked at isolates from rainbow trout. In general the geographic location seems more important than the fish species.

Olga Haenen: Will your sequences be put on fishpathogens.eu?

Søren Kahns: Yes, after publication.

Katja Einer-Jensen: Maybe strains were eradicated because they were stamped out?

Søren Kahns: Yes, it is possible. In 2009 all farms were stamped out at the same time. However, in earlier times, stamping out was not necessary performed synchronous and we cannot tell exactly.

Stephen Feist: Have you looked at your data for the presence of a molecular clock?

Søren Kahns: We have not looked into this in this study.

Katja Einer-Jensen: In a previous publication we addressed the question on when the different genotypes separated from a molecular clock perspective.

CHARACTERISATION OF POLISH VHSV ISOLATES (2005-2009)

Reichert M. [2], **Matras M.** [1], Kahns S.3, Jonstrup SP[3], Olesen NJ[3]
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Abstract:

Abstract: During 2005 – 2009 viral haemorrhagic septicemia virus (VHSV) caused substantial losses in Polish rainbow trout farming. Phylogenetic analyses were performed on 24 selected VHS outbreaks that had occurred in 19 farms. The full G-gene sequences of the VHSV isolates were successfully obtained and analyzed together in with the sequences available in the database www.fishpathogens.eu. Phylogenetic analyses were conducted by the multiple alignment method using the CLUSTAL W program. The analyzed sequences originated from isolates of VHSV collected in the years 2005 – 2009 at different farms in Poland where VHS cases were reported. Eight of these isolates were obtained from three farms (four isolates from two farms collected 2 consecutive yearswith and 4 isolates from one farm collected four consecutive years). The alignment results revealed that some isolates were identical despite quite long distances between the farms from where they were isolated. All remaining VHSV isolates showed varying degree of differences in nucleotide sequence. Altogether it was concluded that the Polish VHSV isolates all belong to genotype Ia like all other freshwater European isolates. But within the Ia group it was possible to divide the isolates into two subgroups: a very homogenous Ia-1 (Pol I) group and a more differentiated Ia-2 (Pol II) group.

Minutes:

Phylogenetic study of 24 VHSV isolates from Poland. Isolates could be divided into two subgroups. Isolates group closely with several German isolates revealing that VHS transmissions across the border between the two countries might occur.

Questions:

Britt Bang Jensen: In the farm where you had reoccurring outbreaks was it the same isolate reappearing or different introduction?

Marek Matras: The 3 of 4 were very similar and could be the same isolate reoccurring while the last was quite different. We will sequence a few more isolates to have a better idea on what is going on.

Guiseppe Bovo: This is a general question to the molecular experts. When you isolate a virus where the disease is endemic should you clone the virus to be sure only one isolate is present?

Soren Kahns: Due to the quasispecies nature, cloning of VHS viruses might not always provide a true picture of the VHSV population. Sometimes you will be able to identify presence of more viruses in a population by analyzing the pictogram of your sequences as multiple double peaks indicate the presence of more isolates

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CHARACTERISATION OF GERMAN IHNV AND VHSV ISOLATES

Heike Schütze
Friedrich-Loeffler-Institut
Federal Research Institute for Animal Health Germany

Abstract:

The fish-pathogenic rhabdoviruses of Infectious Hematopoietic Necrosis (IHN) and Viral Haemorrhagic Septicaemia (VHS) cause substantial losses in German aquaculture. The control of viral pathogens requires intensive studies about the characterization and identification of the respective agent. Phylogenetic analyses permit a clear identification of isolates and their evolution as well as the tracing of trading practises.
Last year 24 VHSV and 5 IHNV outbreaks were reported in Germany. Most isolates from Germany were characterised by sequencing analyses of the glycoprotein gene. Analysed German VHSV isolates are clustered within genotype I group and are closely related to isolates from the last 10 years.

Minutes:

Study of recent German IHN and VHS outbreaks reveals evolution of 0-6 nt/year for IHNV and 0-2 nt/year for VHSV. Spreading and evolution of viral strains in Germany was tracked and relationship between German isolates and foreign isolates was investigated revealing possible transmission across borders. One of the isolates made were from burbot (*Lota lota*) feed on trout diet

Questions:

Olga Haenen: Have you looked at your data in the context of clinics and mortality of the outbreaks?

Heike Schütze: No, here we have represented only the phylogenetic analyses.
Nevertheless, all new IHNV and VHSV outbreaks in Germany are registered in the TSN (= Epizootic News Service). The documents include the respective features, like affected species, clinical signs and mortality.

Stig Mellergaard: Have you analyzed trading relationship between the infected farms?

Heike Schütze: We do not have enough information to confirm any relationship. But from the dataset it seems very likely that a relationship exists.

Katja Einer-Jensen: In USA low evolution rates for IHNV and high for VHSV was observed, you seem to observe the opposite?

Heike Schütze: Yes, and I have no explanation for this.

VIBRIO INFECTIONS IN DUTCH FISH CULTURE

O.Haenen*, I. Roozenburg-Hengst, M. Voorbergen-Laarman, M. Engelsma
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Abstract:

In aquaculture, *Vibrio* infections are diagnosed often. The most important species described in fish are *Vibrio ordalii, V. anguillarum, V. damselae, V. vulnificus, V. alginolyticus, V. parahaemolyticus, V. splendidus – V.lentus* related group*, V. cholerae*, and *V. harveyi*, but also *Aliivibrio fischeri,* and *Aliiv. salmonicida.* Recently, more species are described, like *Vibrio scophthalmi* and *V. ichthyoenteri*, which are genetically related and sometimes difficult to distinguish. *V. scophthalmi* was described as gut bacterium in turbot, and is not in all cases pathogenic. *V. ichthyoenteri* is known to be pathogenic to young flatfish. Vibriosis occurs at low and higher water temperatures, but growth is enhanced at higher temperatures, and in more or less salty water. Some *Vibrio*-species are zoonotic, like for example *V. vulnificus.*

Dutch fish culture occurs mainly indoor, in warm water recirculation farms, some of which are brackish. In The Netherlands, vibriosis has been found for decades mostly in eel, but last few years some cases of vibriosis in newly cultured fish species were diagnosed, like turbot, sole, barramundi and sea bass. Mortalities were various. The affected fish cannot be placed on the market. Antibiotic treatment is no option because of residues in the fish meat.

Vibrio scophthalmi/ichthyoenteri has been isolated a few times from young sole, with fin and muscle haemorrhages, red ulcers and fin rot, and a chronic mortality of 20-30%. Young turbot showed spiral swimming behaviour, petechial haemorrhages in skin and fins, brown red gills, and a high mortality. Another case in turbot resulted in both *Vibrio ichthyoenteri* (from internal organs) and *Pseudomonas anguilliseptica* (from the skin). These fish were lethargic, pale, and had a pale spleen, liver and kidney.

Vibrio vulnificus was isolated several times from eel with severe disease. Additionally, it was found in combination with *Vibrio cholerae/mimicus* in young barramundi, with a red mouth, exophthalmus, tumbling swimming behaviour, and strong fin and gill rot, and a high mortality.

Vibrio anguillarum was isolated from juvenile turbot and sole. Diseased turbot had *V. anguillarum/ordallii* at the skin. Clinics in turbot of 25 cm were superficial and deeper skin lesions with haemorrhages, exophthalmus, fin rot and mortality, by *V. anguillarum*. Sole of 12 cm showed haemorrhages in skin and fins and a chronic mortality, by *V. anguillarum*.

From adult diseased sea bass, *Vibrio harveyi* was isolated. The sea bass showed a grey skin layer, lethargy, anorrhexia, blindness with exophthalmos and a red mouth.
Furthermore, in the past several cases of non typable *Vibrio* species in brackish and marine fish species were diagnosed. These fish showed in general: haemorrhages in skin and fins, with ulcers, and mortality.

It is concluded, that new cultured brackish and marine fish species bring new *Vibrio* infections with disease. It is recommended, to look internationally into vibriosis problems in fish culture, and to improve diagnostic methods for (sub)typing *Vibrio* species.

Minutes:

Presentation on problems with *Vibrio* infections in the Netherlands. The problem is increasing and new cultured brackish and marine fish species cause new *Vibrio* infections with disease.

Questions:

No questions.

SESSION II: Technical issues related to sampling and diagnosis

Chair: *Stephen Feist/ Richard Paley*
Minutes: *Helle Frank Skall*

THE NEW EU MANUALS ON SAMPLING AND DIAGNOSTIC PROCEDURES AND THE ROLE OF THE WWW.EURL-FISH.EU WEB PAGE

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

Abstract:

The final working paper of the Comission decision is still in process and has not been officially adopted yet. The new Commission Decision on sampling and diagnostic procedures will cover molluscs, crustaceae and fish within separate annexes and cover both exotic and non exotic diseases. The methods that were put on the EURL webpage will be closely linked to the methods described in the OIE guidelines, with modifications when judged necessary. The structure of the parts for the specific diseases will follow a common frame I: Description of the aetiology. II: Provisions on how to obtain and maintain certain health status; surveillance programs, eradication, maintaining and regaining disease free status. III: Diagnostic methods; sampling procedure, methods for surveillance in disease free areas and methods for surveillance/diagnostics in endemic areas or if suspicion of disease.
For fish the decision will comprise: VHS, IHN, KHV, ISA, EHN and EUS

VHS and IHN
The diagnostic procedures for VHS and IHN are as you know them.
During transport the samples should not exceed at temperature higher than 10°C and ice should still be at least partly present in at least one of the freeze blocks at arrival at the laboratory.
The virological examination should start as soon as possible and no later than 48 h after sampling. In exceptional cases this can be extended to 72 h.
In case of practical difficulties freezing for up to 14 days of organ material in cell culture medium at -20°C can be allowed. Likewise is it allowed to freeze the supernatant after homogenisation for up to 14 days at -80°C in case of e.g. incubator breakdown.
Culture shall be performed on BF-2 or RTG-2 and either EPC or FHM cell lines. At inoculation the cell lines shall be young and actively growing.
The end dilution of inoculation shall be 1:100 and 1:1000, respectively and at least 2 cm^2 of cell area for each dilution; this corresponds to the well size in a 24 well plate. Subcultivation after 7-10 days and final reading again after 7-10 days. Toxic effects should not be present at final reading.
If cytopathic effect is observed identification of VHS or IHN shall be done by neutralisation, IFAT, ELISA or RT-PCR.
Applied diagnostic methods to rule out and confirm VHS and IHN are I: VHSV/IHNV isolation in cell culture. II: VHSV/IHNV detected in tissues by immunoassay. III: VHSV/IHNV detection by RT-PCR followed by sequencing. Disease is considered confirmed if one or more of the diagnostic methods are positive for VHSV or IHNV. Confirmation of the first case of VHS/IHN in an area previously not infected shall be based on method I, whereas diagnosis based solely on method II or III only applies for endemic areas.

EHNV

The CRL diagnostic manual for EHN differs slightly from the manual presented by the OIE. The differences are based on a cost-benefit analysis. If we can't use the same procedures as we use for VHS and IHN it will cost an enormous amount of money to survey for this disease.

The OIE reference laboratory recommends kidney, liver and spleen. As 2 of these organs are already included in the samples taken for VHS and IHN, we believe it will be OK to use these samples for both purposes, no need for collecting liver.

In order to investigate the growth preferences for EHNV the reference isolate was titrated and incubated at 10°C, 15ºC, 20ºC, 24ºC og 28°C, respectively. The trays were read after 3, 5, 7, 10 and 14 days. In conclusion the final titre is the same in the temperature interval 15-24°C, but titre rise is fastest at 20-24°C.

In order to examine which organs are best to use for isolation of EHNV and to examine if the culture method used for VHS/IHN is acceptable trials were performed at DTU.Vet:

The single most suitable organ for reisolation of EHNV was kidney and regarding number of reisolations the 2 x 7 days method with incubation at 15°C is not the optimal method for detection of EHN, as only 53% of the positive fish were tested positive that way. But since the diagnostics is based on several fish, preferably with symptoms it was concluded that the method already used for VHSV/IHNV is appropriate also for EHNV even though it differs from the OIE manual.

For confirmation of EHNV in case of CPE, IFAT and PCR followed by sequencing of amplicon is used. However one should be aware that the available antibodies against EHNV cannot distinguish between this and other RANA viruses.

KHV

The diagnosis of KHV is primarily based on PCR analysis either directly on fish tissue material or from supernatant from infected cell cultures.

Following several proficiency tests and workshops the most suitable methods for detection of CyHV-3 was decided and included in the diagnostic manual posted on the EURL web site.

One of the major concerns are the number of false positive and false negative results obtained in the various ring tests, e.g. during the CEFAS KHV ring trial 10 of 44 laboratories produced false negative and 19 of 44 laboratories produced false positive results!

EUS

The Oomycete *Aphanomyces invadans* is regarded as the causal agent of Epizootic Ulcerative Syndrome (EUS).

A suspect case of EUS according to the OIE diagnostic manual 2009 is related to typical lesions in susceptible fish or presence of *Aphanomyces* sp. without further identification. A confirmed case is a suspect case presenting typical mycotic granulomas in tissue or where the agent has been identified by PCR or FISH detection techniques in tissue or where *A. invadans* has been isolated and confirmed by either bioassay, PCR or sequence analysis.

Sampling should be done from various lesions and organs and should be done in the edge of a lesion from a clean cut surface. A variation in pathology and clinics is seen in between cases, especially formation of granulomas cannot always be expected - this depends upon variable factors such as stage of infection, age and type of fish etc. There are three PCR methods published "Oidtmann", "Vandersea" which is specific and "Phadee" which can cross react with other *Aphanomyces* spp. and which also don't amplify all strains of *A. invadans*. Of the Oidtmann and Vandersea, Oidtmann produces a somewhat longer PCR product which is suitable for sequencing. The methods are

finalised and will be uploaded on the EURL web page after final revision. The methods will be described by Club 5, with Dr. Olga Haenen, CVI as coordinator.

ISA
The diagnostic methods are almost the same as the methods described in the latest edition of the OIE manual. The criteria for diagnosis will most likely also include sequencing in order to assess the HPR type of the isolates or alternatively to use HPR0 specific real-time RT-PCR for all findings from fish without clinical symptoms.

Minutes:

The presentation focused on the diagnostic manuals that will be issued. The Commission Decision 2006/88/EC was decided in 2006 and should have been fully implemented by 2008. This has unfortunately not been the case with regard to diagnostic procedures for the listed diseases that has not yet been described and we have to rely on the former 2001/183 for VHS and IHN, and 2004/466 for ISA. There exists no diagnostic manuals for EHN, EUS or KHV and such have to be made.
The new CD is still in preparation and comprises all listed diseases in fish and molluscs. Detailed diagnostic procedures will be put on the EURL web pages.
The template for VHS and IHN will also be used for the "new" diseases.
Surveillance programmes: The quick model, model A, is similar to the one described in the OIE manual with a large sample size for 2 years. In model B the surveillance programme will last for 4 years with a small sample size. Model B will often be used in areas where you have had the disease endemically and I find this model much more valuable in endemically areas as the virus can hide in the environment for at least up to 4 years, as shown in Søren Kahns' presentation earlier today, whereas model A is usable where you get an outbreak in a free areas as in the UK case.
Targeted surveillance is obligatory in cases where the whole country is not free of the disease in question. Also if no clinical symptoms will be present, e.g. ISA in rainbow trout, targeted surveillance is also necessary.
For VHS, IHN and EHN only isolation of the virus is necessary to demonstrate existence, clinical disease is not necessary.
I am quite confident that in the future it will be possible to survey for VHS and IHN by real-time RT-PCR but we need to have validated methods before including real-time RT-PCR in the diagnostic manual. There have been problems with sensitivity and specificity for this method which we have not had with cell culture.
For ISA we have problems that not all isolates are easy to cultivate. For HPR0 it has not been possible and even the high virulent isolates can be difficult to cultivate.
For KHV, surveillance samples should not be pooled, and pooling is only advisable in diseased fish and then from max. 5 fish.
For EUS, in the OIE manual clinical symptoms only are enough to state presence of the disease. I am not sure if this is the correct method and believes that the identity of the pathogen by e.g. PCR should be tested.
At present, manuals for VHS, IHN, EHN and ISA have been uploaded on the EURL webpage but not approved by the Commission.

Questions:

Alexandra Adams: For KHV will serology be included?

Niels Jørgen Olesen: For VHS and IHN we will hopefully be able to include serology as we are close to finish the validation for these two diseases. But for KHV we do not have a validated method yet.

Alexandra Adams: With KHV we sometimes find strange results with PCR and it would be nice to back up with serology.

Niels Jørgen Olesen: Yes, I think we all see these strange results sometime and how we deal with this will have to be solved.

Brit Hjeltnes: Why is it enough for some diseases only to isolate the virus whereas for others you also need clinical signs of the disease?

Niels Jørgen Olesen: I think this is based on historical reasons. For VHS the surveillance for the diseases was started when the causative virus was isolated whereas for ISA the surveillance was started based on clinical symptoms. For VHS, finding of the virus was reason for expecting presence of the disease as all isolates at that time were pathogenic.

DIAGNOSTIC METHODS FOR IDENTIFICATION OF EHNV AND OTHER RANAVIRUSES

Heike Schütze
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Federal Research Institute for Animal Health Germany

Abstract:

Ranaviruses have been isolated worldwide from fish, amphibians and reptiles at an increasing frequency over the last few decades. They cause systemic infection with necrosis of kidney and spleen as well as diffuse subcutaneous and internal haemorrhages and induce high mortalities in host species. Since 2006/2009 the epizootic heamatopoietic necrosis (EHNV) and ranavirus infection of amphibians are listed diseases of the OIE and EU.
Recommended methods for the diagnosis of EHNV are summarized under section 2.3 of the OIE manual. The manual for amphibian ranaviruses is in preparation. Using serological techniques an identification of most ranaviruses is possible. Nevertheless a differentiation has failed. Molecular techniques facilitate the identification as well as the differentiation of ranavirus isolates. Different methods for PCR and restriction enzyme analyses were compared and evaluated. A new method was established to detect and differentiate all published ranaviruses with the exception of the Grouper iridoviruses.

Minutes:

The history of ranavirus started with isolation of frog virus 3, which became the type species.
EHNV and other ranaviruses are extremely resistant. The virus can be inactivated by treatment with 70 % ethanol or with sodium hydrochlorite or by heating to 60 °C for 15 minutes. Recommended cell lines for EHNV propagation are: BF-2, FHM, EPC and CHSE-214. The virus replicates at 15 – 20 °C.

Ranaviruses do not induce neutralising antibodies. Using the available and recommended rabbit or sheep anti-EHNV sera (OIE Manual), a differentiation is not possible, means it is only an identification of a ranavirus isolate. These sera cross react within most ranaviruses with exception of the Santee-Cooper ranaviruses and the grouper iridoviruses.

The MCP is highly conserved within family *Iridoviridae* and also in other viral families. Within an EU project we have identified the complete MCP gene sequence from various ranavirus isolates. Sequences from some isolates were completely or partially available. The aim of this work was to establish a diagnostic tool for identification and differentiation of most ranavirus isolates including the Santee Cooper ranaviruses.

Known and new identified sequences of the MCP gene from several ranaviruses were compared.
The phylogenetic tree demonstrates the closed relation between most ranavirus isolates. The Santee Cooper ranaviruses LMBV, GV6 and DFV cluster in a separate group. The Grouper iridoviruses SGIV and GIV are the most distant from the others.

Based on the sequence alignments a new PCR called Rana MCP PCR was established and validated using 12 different ranavirus isolates. The resulting product is 625 bp in length. After digestion with the restriction enzyme Sal I, EHNV can clearly be discriminated from other ranaviruses.

Some remarks for further discussions: With exception of the grouper iridoviruses and the Santee Cooper ranaviruses all ranaviruses are very strong related. Why EHNV was listed by OIE and the European commission? We have outbreaks of ESV and ECV in farmed sheatfish and catfish in Germany, Italy and France.
Furthermore, within the EU project RANA we have shown that European farmed perch and rainbow trout are not susceptible for EHNV. So a further discussion about listed diseases is indicated.
A final question that I would like to raise: Should all ranaviruses be listed or should EHNV be excluded from the list?

Questions:

Neil Ruane: Will this method of yours be published?

Heike Schütze: Yes, the manuscript has been accepted.

Niels Jørgen Olesen: Remember that in the proficiency test provided by the EURL, detection of ranavirus is included and it might be possible to include the RFLP. Heike, do you think that all ranaviruses should be included or should EHNV be excluded?

Heike Schütze: I think EHNV should be excluded. Outbreaks of similar diseases induced by very closely related ranaviruses e.g. ECV and ESV were observed in Italy, Germany and France.

DIAGNOSTIC PROCEDURES FOR DETECTION OF *APHANOMYCES INVADANS* – THE CAUSATIVE AGENT OF EUS

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Abstract:

Epizootic ulcerative syndrome (EUS), caused by the oomycete *Aphanomyces invadans (A. piscicida),* is a serious emerging notifiable exotic disease, diagnosed in >60 fish species of various families in Asia, Australia, N-America, and Africa. There is no therapy against EUS, and no vaccine. All EU NRL's for Fish Diseases must have EUS diagnosis in place (ref. 2006/88/EC). Although some tests for EUS were described in literature, most tests were not validated.

The aim of this project was to build up knowledge to recommended confirmatory methods for EUS into the EU:

Try out the described EUS methods (fresh smears, fungus isolation, histopathology, PCR), choose methods for validation, and write Operational Procedures (OP's)
Develop if needed alternative methods (e.g. other agars for isolation, real-time PCR), and validate and implement them.
Establish an electronically available slide collection for EUS histopathology.
Write OP's for the recommended diagnostic tests for EUS based on our findings.
Evaluate methods for storage, survival of *A. invadans* outside the host and suitable disinfection.

The four labs kindly received two reference strains from Prof. Kanchanakhan (OIE Reference laboratory in Bangkok), and from Prof. Hatai, Japan.

Results of this project (2010-2011):

- OP´s for 1) selection of fish to sample, 2) sampling for isolation, 3) sampling for PCR & histopathology, 4) cultivation of isolates of *A. invadans*, 5) sporulation of *A. invadans*, 6) (q)PCR's for EUS, and 7) histopathology of EUS.
- An electronically available slide collection for EUS histopathology.
- Recommendations on storage, survival of *A. invadans* outside the host and disinfection. One important finding was that ethanol is not suitable to disinfect *A. invadans*.

The output (OP's, slide collection, recommendations) can be used by the NRL's for Fish Diseases of Europe, and will be accessible via the EURL Fish Diseases website (www.eurl-fish.eu).
This project was funded by Club 5.

Minutes:

I recommend that if it is the first time you isolate EUS in your country, you should also perform PCR and sequencing to confirm that it is *A. invadans* as it is important to be 100% sure before informing OIE and EU.
SOP's will be put on the EURL webpage in the near future.
Ethanol will not disinfect *A. invadans* but can be used to disinfect the skin when taking samples to avoid other microbes.

Questions/comments:

Sigrid Cabot: The Commission has requested an opinion from EFSA on the risk of introduction, spread of EUS into Europe and probable consequences for such a spread.

Olga Haenen: We look forward to see the result of this.

SPORULATION OF APHANOMYCES INVADANS

Christian Fry
Section for Fish Diseases, Division of Poultry, Fish and Fur Animals, National Veterinary Institute, Technical University of Denmark, DK-8200 Århus N, Denmark

Abstract:

Aphanomyces invadans is an oomycete which is associated with epizootic ulcerative syndrome. Over 50 species of fish have been confirmed by histological diagnosis to be naturally affected by EUS. Many of these fish are commercially important, both in the ornamental fish trade and the aquaculture industry. Diagnosis of *A. invadans* usually based on clinical signs and confirmed by histopathology. Demonstrating typical asexual characteristics by inducing sporogenesis allows identification of the oomycete to the genus level. After inducing sporulation, the zoospores can be isolated for use in clinical infection of fish through subcutaneous injection or bath challenge system. The presentation will include a brief description on optimized techniques for inducing sporulation, and footage obtained during the sporulation process.

Minutes:

Using hemp seeds for sporulation is not included in the OIE manual but seems to enhance the sporulation efficiency as the oomycete will attach to the seed and the seed will not give any nutrition to the oomycete. The washing step is crucial for sporulation as it is important removing all the nutrition to induce sporulation.
An interesting video showing the sporulation in real time was presented.

Questions

Steve Feist: Why does the primary zoospore fly up through the evacuation tube?
Nobody could answer this question.

Richard Paley: Have you compared your method to sporulate with the OIE manual which produces the most zoospores?

Christian Fry: We obtains the best sporulation when increasing time of washing

Niels Jørgen Olesen: EUS was found recently in Canada and probably we will also soon find it in Europe, so it is important to be prepared for diagnosing the disease. As I understand it all exporters have to document freedom for EUS?

Sigrid Cabot: Freedom of EUS have to be documented for susceptible and vector species intended for further farming in the Union, but a derogation from this requirement is currently applying for ornamental fish intended for closed ornamental facilities. The Commission has requested EFSA for an opinion on EUS.

Niels Jørgen Olesen: At the proficiency test for 2011 it is our aim to include EUS. Olga raised the question if we should survey for EUS in Europe?

Regarding reference material for the NRLs I suggest that the EURL (or CEFAS) provide you with reference material for PCR.
How many of you are ready to diagnose EUS? Participants indicated that 5-6 countries were ready to diagnose (Norway, DK, UK, Sweden, Holland, Germany ….)

Olga Haenen: I think especially countries that trade a lot with ornamental fish should be prepared to diagnose this disease. As a lot ornamental fish is imported through Schiphol we are prepared.

Niels Jørgen Olesen: I guess also the Czech Republic trade a lot of ornamental fish.

Brit Hjeltnes: Have anyone done infection trials with this pathogen at fish species present in Europe and using temperatures we have here?

Olga Haenen: Birgit Oidtmann has made experiments at permissive temperatures.

Richard Paley: She has tested mullets and other fish species at 18-22°C and found more tested fish species to be infectable.

Stig Mellergaard: If we get the disease, will we ever be able to eradicate it? How much effort should we put into it?

Olga Haenen: I doubt eradication will ever be possible in the wild. We cannot say that we are 100% free of the disease as we have not examined it.

Niels Jørgen Olesen: If we find it in aquariums it will be possible to eradicate it.

Sigrid Cabot: We need to have an assessment of possible routes of introduction into Europe and the risks of the disease to spread within the Union. If the disease is endemic we would have to reconsider the listing of the disease. Since it is a notifiable disease at least a passive surveillance system should be in place in all Member States.

Olga Haenen: Regarding passive surveillance we talk mainly about closed facilities as we will not be able to do passive surveillance in the open waters.

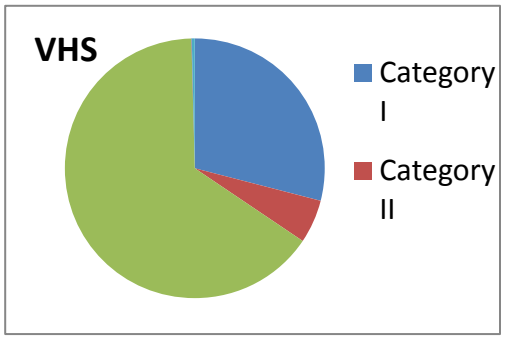
Niels Jørgen Olesen: You will see lot of fish with similar symptoms to those you have seen on the photos today, but as people have not been aware of the disease and the diagnostics have not been in place people have not looked for it. Discussions and distribution of information about the disease should raise the awareness.

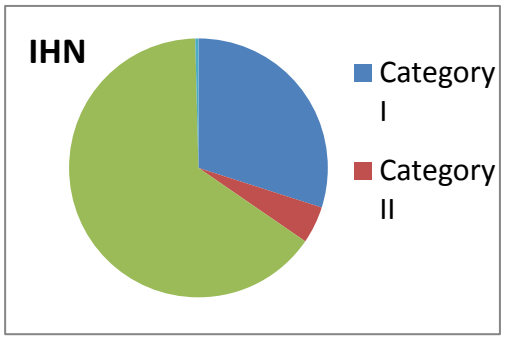
HEALTH CATEGORISATION OF FISH FARMS IN EUROPE IN 2010

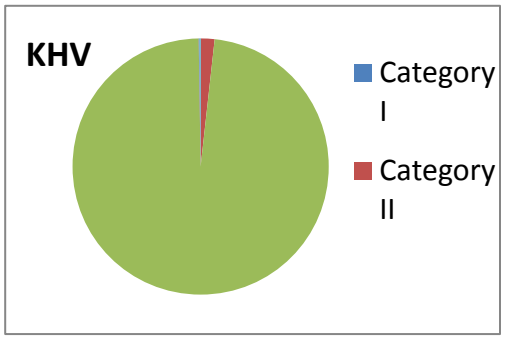
N. J. Olesen and N. Nicolajsen
National Veterinary Institute, Technical University of Denmark

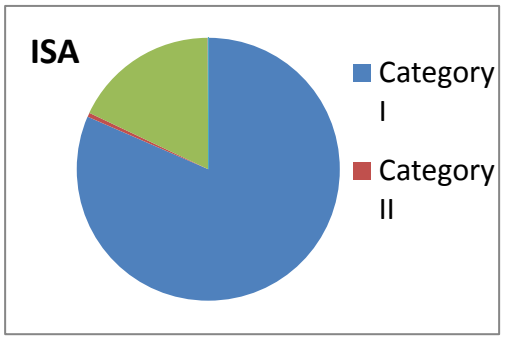
Abstract:

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.
More than half of the authorised farms in Europe are in category III for VHS and IHN and the remaining in category I or II. According to these official data almost no farms are infected with either of these diseases. This might be more due to a significant underreporting than of the de facto situation.
For KHV most carp farms are in category III, unknown status.
Many farms in Europe are not categorised yet. However, categorisation is in good process when comparing to e.g. the molluscs farms in Europe. There are several different views on how categorisation shall be performed, e.g. should VHS free marine rainbow trout farms be placed in cat III or I? If ISA virus HPR0 is found in or in proximity of a farm can it remain its cat. I status? The Council Directive is under revision and in this connection the categorisation system might be simplified and be made more transparent.









Minutes:

The health categorisation process in Europe on fish farms is well in progress. The lack of farms in cat 4 and 5 for VHS and IHN most definitely is a question about under-reporting. The high number of farms in category 3 might be explained by the transitatory state the categorisation is in, where difficulties in how to handle the interaction between farmed fish and wildlife with low pathogenic variants of pathogens e.g. ISA HPR0 can be seen. Furthermore this has driven a shift in how category 3 is seen by member states, where category 3 has shifted from an interim category, to a category including uncertainties.

Questions:

Stig Mellergaard: The categorisation in the area of aquaculture lacks simplicity compared to terrestrial animals. Categories could with benefit be reduced to 3; infected, fallowing in process and free.

Sigrid Cabot: In the framework of the Animal Health Law the issue of 3 or 5 categories is being discussed.

CHALLENGES REGARDING IMPLEMENTATION OF THE NEW LEGISLATION ON AQUATIC ANIMAL HEALTH SURVEILLANCE IN EUROPE

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Abstract:
In order to keep up to the SPS-agreement and new knowledge on risk-based surveillance, a new Council Directive (CD) on aquatic animal health surveillance and control was adopted (CD 2006/88/EC) in 2006. One of the intentions of this CD was that disease control should be more cost-effective and risk-based, and focus more on prevention than control. All aquaculture production businesses (APBs) must be registered and authorized, in order to enable an early warning system, with detection of increased mortalities. The risk-based surveillance system for listed diseases must be based on risk-ranking of APBs according to an evaluation of their risk of contracting and spreading disease, and their current status of infection.
In this study, we have asked aquatic animal health professionals in the European Countries how the status is for implementing the legislation in their home country, and what challenges they face in relation to this. A questionnaire was distributed to 34 countries, of which 25 responded.
The survey revealed that there have been delays in establishing registers of APBs, due to the complexity of the industry and lack of resources. 60-80% of the responding countries had ranked farms according to health status, but only 4 countries had drawn up a surveillance program. Furthermore, only 4 of the 25 countries had drawn up contingency plans for handling of exotic and emerging diseases. None of these had yet been approved by the EU.
Thus, many European countries have yet to implement the new legislation concerning aquatic animal health surveillance. Several countries are experiencing problems with inherent complexity of the legislation and the organisation of their production and thus the specific requirements that relate to their within-country scenario. It seems that both in the EC and many of the MS, aquaculture is not prioritized as opposed to terrestrial animal farming.
Thus, as of now, the general aquatic animal health has not yet benefited much from adaptation of new legislation.

Acknowledgements
All respondents are acknowledged for their contribution by answering the questions and providing their opinions in the survey.

Minutes:
The preceding animal health legislation concerning aquatic animals lacked that it was not risk based and that it was not flexible. As of the Council directive 2006/88/EC this should be of more modern animal health program based on: 1) registration/authorisation 2) risk based surveillance and 3) contingency plans. Though good intentions, following the results in our questionnaire there is some way to go to full fill the directive. In most member states initial steps to address the directive have been done, but finalisation of surveillance programs and getting contingency plans approved in the EU has not yet been done. The main problems raised in our questionaire are the complexity of the industry and the lack of dedicated time and resources for implementation.

Questions: No questions.

DEVELOPMENT AND ASSESSMENT OF A REAL TIME LAMP ASSAY FOR KHV

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Abstract:

Loop mediated isothermal amplification (LAMP) is a relatively new and simple method for the amplification of DNA (Notomi *et al*., 2000). The process requires 4 or 6 primers which can provide high specificity. Two of the primers are designed to form loops and are thus self priming meaning the reaction proceeds rapidly and can occur at a single temperature leading to simple and cheap equipment requirements. A number of LAMP assays have previously been developed for Koi herpesvirus (Gunimaldevi *et al*., 2004; Soliman and Matbouli 2005 and 2009; Yoshino *et al*., 2006) with varying sensitivities and length of time to result and all requiring gel electrophoresis or a rather imprecise in tube visual detection. OptiGene Ltd (UK) has developed a novel recombinant polymerase in a proprietary mastermix and an instrument (Genie) for LAMP amplification and analysis. The novel enzyme is reportedly faster than currently used enzymes and the Genie instrument incorporates heating blocks and a fluorimeter providing the conditions for LAMP amplification and allowing the monitoring of these amplification reactions in real time. Furthermore melting curve analysis can be performed on the amplification products to interrogate specificity. We compared a published LAMP assay and newly developed assays using the Genie instrument and OptiGene reagents against nested PCR and realtime qPCR assays on 174 archived diagnostic samples for the presence of KHV. The new LAMP assay detected 10^5 copy number positive control template in a background of negative DNA in less than 10 minutes. The assay consistently detected down to 50 template copies in a reaction from diagnostic samples and occasionally detected as low as 10 copies. There was evidence of some cross reaction with other herpes viruses but not with other non-related viruses. The assay was broadly speaking equivalent to the nested PCR assay with TK specific primers, which is more sensitive than the single round PCR currently recommended by the OIE reference laboratory for KHV detection. Taking PCR as the "gold standard" the LAMP assay showed 89.4% specificity and 86.5% sensitivity with the nested PCR. The LAMP assay is simple and rapid to setup, appears as sensitive as nested PCR, may be less prone to contamination and provides rapid diagnosis and easy interpretation but the potential cross reactivity needs to be assessed further.

Minutes:

LAMP assays have been reported for KHV but are not widespread due to practical issues. We have tested a new commercial real time LAMP method using a small portable machine both to run the amplification procedure and to analyse the product outcome. The initial tests of the system revealed problems with the procedure, but after optimisation done by the commercial company 174 samples were analysed. The test revealed nice results and only a few false positive which was most likely cross contamination. A big advantage with the LAMP procedure is that the specificity can be investigated by analysing the melting point on the products. The overall performance of the test was promising especially as a field tool. Since it was rapid and with comparable sensitivity and specificity as PCR methods.

Questions:

Søren Peter Jonstrup: Would you skip the PCR over LAMP?

Richard Paley: PCR and sequencing still have a big and important role. The LAMP is especially good for use as a quick diagnostic tool.

Stephen Feist: In the future, will we just sequence everything?

Richard Paley: Currently this is still not cost effective enough. However, it might be a possibility in the future.

FISH CELLS – SOME REMARKS TO INDUCE DISCUSSION

Heike Schütze
Friedrich-Loeffler-Institut
Federal Research Institute for Animal Health Germany

Abstract:

A uniform standard, reproducibility and clear references are essential for diagnostics. Sampling plans and diagnostic methods for the detection and confirmation of certain fish diseases are defined in decision 2001/183/EC. Diagnostic methods are also recommended in the diagnostic manuals of the OIE. For the propagation of fish viruses different cell lines are recommended. The development of molecular techniques facilitates the identification of cells and conclusions about their origin. Based on two examples the importance of a clear declaration of cell lines will be demonstrated. The EPC is susceptible to most fish viruses and particularly suited for diagnostics. Genetic analyses have shown that the EPC line is an epithelial cell from skin of fathead minnow and not from carp as originally assumed and published. Based on the activities of the FLI Germany the RTG-2 cell is recommended by the EU (2001/183/EC) for propagation of VHSV. This cell line with cell number CCLV Rie 0088 originates from bluegill fry and not from rainbow trout gonads. Nevertheless, the CCLV 0088 redefined as RT/F is recommended for propagation of VHSV. To establish a uniform standard in all National reference laboratories a clear definition of recommended cell lines is essential. The cell culture library of the FLI Germany provides service and support.

Minutes:

To harmonise diagnostic works it would be advantageous to use cell lines with explicit history. The EU decision from 2001 regulates the sampling plans and diagnostic methods for detection and confirmation of certain fish diseases. Recommended cell lines for diagnostics of IHNV, VHSV, and IPNV are BF-2 or RTG-2 and EPC or FHM cells. Recent evidences have shown, that origin of cell lines can be questioned. If we use and include the catalogue or reference number in OIE documents we have a clear definition of used cell lines.

1. Example: EPC line is an epithelial cell from skin not from carp but from fathead minnow. Since this year EPC is available from ATCC (CRL-2872). Cells were deposited by Winton, 1969 is given as year of isolation and under comments the inconsistency is clarified. In summary: Do we use all the same EPC?
2. Example: Based on the activity and the recommendation of FLI in Germany the RTG-2 cell, listed in our cell library under the number CCLV Rie 0088, was included in the decision 183 from the European Commission for diagnostic of VHSV. But this cell line (CCLV 88) has its origin not from rainbow trout gonade as assumed, but from bluegill fry. In consequence our cell library has declared this line under the abbreviation RT/F. The catalogue number CCLV Rie 0088 remained unchanged. It should be remark, that this cell line is different from BF-2.

A clear declaration of cell lines based on reference numbers/ certification number is essential. The origin of cell lines is not always well defined. Information of the origin of a cell line is based on the respective or actual knowledge we have. Different cell lines exist from same host, but they represent different cell lines or sublines. And, various sublines from the same or similar origin exist in different labs. In conclusion, different results will be achieved. But for diagnostics we need a unique standard, with reproducibility, and clear references.

It is a scientific practice of some journals to proof the reference and certification of used and described cells.
The FLI offers following: FLI will buy EPC, RTG-2 and BF-2 cells from ATCC. These cells will be cultivated and proofed in our cell library. The NRL Germany will check these cells in comparison with other cells for the susceptibility of different viruses. If all is ok, the cell library will send these cells to the EURL and NRL's. Everyone will receive the respective certificate for the cells. EURL and NRL pay only for the transport. The NRL's send this defined material to their regional lab's. At the end we have unique standardized cells in all reference lab's.

Questions:

Brit Hjeltnes: Are you questioning the ATCC?

Heike Schütze: No.

Brit Hjeltnes: Why then, won't the cells just change over time anyway?

Heike Schütze: Every lab has their own cell lines and sub lines. Based on new techniques the origin of some cell lines was identified and corrected. If we talk about a cell line, possibly we talk about different cells or sublines. As NRL we need a standard with well defined cells including the certificate number.

Niels Jørgen Olesen: Thank you for your generous offer which I am sure could be of help for some laboratories. However I think the most important issue here is the sensitivity of the cells, which has been a key issue in sending out the proficiency test where participants can check and standardise sensitivity of the cells used in Europe for diagnostic work. If your cells are not sensitive enough, the EURL can send you cells that are sensitive.

Guiseppe Bovo: I agree with Niels Jørgen. Central acquired cell lines are not better. I have experienced buying cells which from the ATCC had been treated for Mycoplasma, these cells could not be used for diagnostic work. The way to go is getting uniformity in sensitivity among the NRL's by the mean of the proficiency test.

Helle Frank Skall: Getting cells with the same reference number is not a guarantee for similarity as sensitivity can change when cells are passaged.

PITFALLS AND CHALLENGES IN DEVELOPMENT OF REAL-TIME PCR DIAGNOSTIC ASSAYS

SP Jonstrup[1], S Kahns[1], M Christophersen[1], NJ Olesen[1]
1 Section for Fish Diseases, Division of Poultry, Fish and Fur Animals, National Veterinary Institute, Technical University of Denmark, DK-8200 Århus N, Denmark

Abstract:

Late January to early February 2011 a training course was conducted here in Aarhus with participants from all over Europe. Here we had many fruitful discussions on PCR related topics and it was clear that a lot could be learned by sharing non publishable experiences from different laboratories. In this talk I will share some of this knowledge with you. Real-time PCR as a tool for doing diagnostics of fish diseases is getting more and more common. There are many advantages of this technique. It is fast, sensitive, and if designed properly very specific. Compared to conventional PCR there is no need for opening the tubes post PCR, thereby lowering the risk of contamination. In real-time PCR the Ct (cycle threshold) value is defined as the number of cycles required for the fluorescent signal to cross the threshold (i.e. exceeds background level). If two samples in the same assay is compared the sample with the lowest Ct value is regarded most concentrated and if no Ct value is obtained the target is regarded below detection limit. However in some cases interpretations based solely on Ct values can lead to mistakes. This talk will be a presentation of some of the pitfalls we discovered during our investigations and will hopefully illustrate how one should be careful about relaying only in the information contained in the Ct value.

Minutes:

One has to look into the background of the obtained CT values. One should set the baseline of thresh-hold and background signal according to what is appropriate in your set of samples. We have experienced problems with primer dimer which were difficult to see but caused inconsistency in results. By running the samples using Cyber Green instead of Taqman probes we could visualise this problem allowing us to make corrective measures and re-design our protocol whereby the Taqman probes were running as expected. Buffers and kits and commercial products can vary in efficiency, in our newly developed real time RT-PCR detecting all VHSV genotypes we saw that Qiagen kits performed up to 7 Ct values better than Biorad and Stratagene kits. This might be due to that the Qiagen kit can handle small mismatches in probe binding sites better than the others. However, if this is the reason that the Qiagen kits performs better in our assay, then it might not always perform better compared with the other depending on the design of your assay.

Questions:

Debes Christiansen: You can't shift your threshold in the software on a daily basis. However, this must be standardised and uniform between runs.

Søren Peter Jonstrup: All systems include variables, such as machines, days, technicians etc. Therefore it can make sense to look into how your assay has performed and whether the default settings can handle how the assay has been running.

SESSION III: Scientific research update

Chair: *Søren Kahns*
Minutes: *Søren Peter Jonstrup*

INFECTIOUS SALMON ANAEMIA (ISA) IN NORWEGIAN SALMON FARMING, A CHRONICLE OF EVENTS RELATING TO A SMALL SCALE EPIDEMIC

E. Karlsen[a], E.J. Johansen[a], T.M. Lyngstad[b] and P.A. Jansen[b]
[a] The Norwegian Food Safety Authority, Pb 383, 2381 Brumunddal, Norway
[b] National Veterinary Institute, Pb 750 Sentrum, 0106 Oslo

Abstract:
ISA is a viral disease of Atlantic salmon which is subject to a strict control regime in Norway enforced by the Norwegian Food Safety Authorities (NFSA). The annual number of confirmed ISA-outbreaks in Norwegian salmon farms have varied from 1 – 23 during 1993 – 2009. Outbreaks have partly emerged isolated from other outbreaks in space and time, but partly also appeared in small space-time clusters. The aim of the present study was to compile a detailed database describing salmon farming operations and disease progression in a local area experiencing a small scale ISA epidemic. We present a chronological history of events relating to farmed salmon populations during 2007 – 2010, and a total of 22 ISA outbreaks in these populations, occurring in a local area in North Norway. The timing of events has partly been compiled from production plans approved by the local NFSA and partly from information gathered directly from the salmon farmers. The progression of ISA in the area rely on reports from the salmon farmers, surveillance of cage-level mortality on affected farms, and official management decisions made by the NFSA. Maps and animations are used to visualise the dynamics of the salmon farming operations, progression of disease and other events in the area. The chronicle shows how the local structure of the salmon farming industry constantly changes due to; i) fish farm sites being phased into or out of production, ii) populations of fish being moved between sites, iii) fish escaping from cages on farm sites, iv) and finally due to the progression of ISA in the area leading to implementation of control measures. The detailed account of salmon farming operations and disease progression serve as a basis for an epidemiological study, in which genotyping of the ISA-virus have been used to trace dispersal pathways for the virus.

Minutes:

A description of disease progression of an ISA epidemic in the north of Norway (2007-2010) was presented. Escape and transportation of fish seems to be involved in spreading of disease.

Questions:

Niels Jørgen Olesen: Did you make any surveillance of wild fish? Did you find HPR0 in the wild fish population? Was there any genetic relationship between any HPR0 isolates and the isolates causing outbreaks?

Einar Karlsen: We have not sampled from wild fish. We have no evidence that the outbreaks originated from a mutated HPR0 virus. We have indications that there might have been a not recognised outbreak to start with and that these fish were moved around in the region.

Brit Hjeltnes: If a bigger area was fallowed to start with the problem could have been solved quicker, but politics and economic interests made this solution impossible.

Einar Karlsen: I am not sure that a quicker fallowing would have solved the problem and this would have had a large impact on the local community making it hard to have achieved.

INFECTIOUS SALMON ANAEMIA AND HPR0 STRAIN – AN OVERVIEW

Eann Munro
Marine Scotland Science

Abstract:

Infectious salmon anaemia (ISA) is a multisystemic contagious disease of farmed Atlantic salmon (*Salmo salar* L.) caused by a single stranded enveloped RNA-virus in the family *Orthomyxoviridae*. The virus (infectious salmon anaemia virus; ISAV) contains eight segments within its genome. The disease is characterised by severe anaemia and haemorrhaging in several organs, including the liver, kidney, gut and gill. Disease outbreaks are predominately associated with Atlantic salmon farmed in the marine environment.

ISAV possess two surface proteins, a haemagglutinin (H) and a receptor destroying esterase (E) which are important determinants of virulence (Kibenge et al., 2007). Multiple types based on a highly polymorphic region (HPR) of the haemagglutinin-esterase (HE) gene located on genomic segment 6 have been reported (Markussen et al., 2008). A variant with a longer HPR type, HPR0, was first detected in Scotland in 2002 (Cunningham et al., 2002). Evidence for the HPR0 viral strain comes from PCR and direct sequencing from fish tissues. The putative strain of ISA does not appear to induce disease in Atlantic salmon and is unculturable by *in-vitro* tissue culture. Virulent ISAV strains are hypothesised to have evolved from HPR0 as a consequence of transmission from a viral reservoir to densely populated Atlantic salmon farms.

Recent work by Christiansen (2011) reported that ISAV-HPR0 appeared as a seasonal and transient infection on Faroese Atlantic salmon farms without associated mortality. They suggest that ISAV HPR0 causes a subclinical respiratory infection more like seasonal influenza, compared to the systemic infection induced by pathogenic ISAV.

Factors governing the maintenance of HPR0 in fisheries and the external environment and its role as a risk factor in leading to the emergence of ISA disease must be better understood.

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Minutes:

Surveillance for HPR0 in marine salmon farms in Scotland revealed occurrence of HPR0 in around 10% of samples. None of the found HPR0 viruses seem to be the ancestor of the ISA outbreaks in Scotland. Around same prevalence was found in the Faroe Islands. Attempts to develop challenge models for HPR0 were so far unsuccessful. To date, the detection of HPR0 RNA has not been

associated with ISA disease. It seems that new outbreaks of ISAV to a very large extend is due to horizontal spreading of pathogenic ISAV and that HPR0 evolution into a pathogenic strain is a rare event. Organizing the industry into management areas in Scotland reduced the risk of long term persistence of pathogens and facilitated rapid containment of new emergences of the disease. HPR0 has a tissue tropism different from pathogenic ISAV, with gills as the primary location of HPR0.

Questions:

Olga Haenen: You have to notify OIE even when you find HPR0. What do you think about this?

Eann Munro: If we only detect by PCR as for the HPR0 strains we do not consider it as ISA and therefore do not report it.

Brit Hjeltnes: In infection trials with HPR0 do you think you get replication?

Eann Munro: We think we can get it to replicate in the gills.

DETECTION OF LOW-PATHOGENIC INFECTIOUS SALMON ANEMIA VIRUS (ISAV-HPR0) IN FRESHWATER IN THE FAROE ISLANDS

D.H. Christiansen,
Food and Veterinary Agency, National Reference Laboratory for Fish Diseases, Tórshavn, Faroe Islands

Abstract:

Infectious salmon anemia (ISA) hit the Faroe Islands in 2000 and the following five years the ISA epidemic spread throughout the islands causing an almost total collapse of the industry. A subsequent restocking program with Atlantic salmon vaccinated against the ISA virus and a comprehensive screening program for ISAV provided a unique opportunity to study the risk of re-emergence of ISA in the Faroe Islands. We recently reported that a Faroese ISAV-HPR0 subtype is widespread in seawater farmed Atlantic salmon and causes a transient and seasonal infection without clinical ISA[1].

In our screening program for ISAV six freshwater farms with continues production of Atlantic salmon smolt and Faroese broodfish were screened for the presence of ISAV. During the study period from February 2007 to December 2010 Atlantic salmon smolt from four of the six freshwater farms showed transient infection with ISAV-HPR0. In total 8,7% apparently healthy smolt tested positive for ISAV-HPR0 by RT-PCR and sequencing. The annual prevalence ranged from 0% ISAV-HPR0+ve in 2007 to 14% ISAV-HPR0+ve in 2010. Furthermore, we found a high prevalence of 48% and 93% ISAV-HPR0+ve broodfish stripped in 2008 and 2010, respectively. Since vertical transmission has yet to be definitively demonstrated, the origin of these infections and thus the potential transmission pathways for ISAV-HPR0 remain uncertain. To disentangle potential transmission pathways we have performed genetic analysis of ISAV-HPR0 isolates from broodfish, freshwater smolts and seawater Atlantic salmon. Our preliminary results will be presented.

Minutes:

ISA caused a collapse of the Atlantic salmon industry in the Faroe Islands but the production has now been re-established. HPR0 virus was detected inside gill cells by IHC. Two major HPR0 genotypes are present in the Faroe Islands. The ISAV isolates from outbreaks in the Faroe Islands is closely related to one of these. HPR0 was also found in freshwater farms. HPR0 infection is low pathogenic and transient but highly contagious.

Questions:

Brit Hjeltnes: Interesting that you find HPR0 in freshwater. What could be the source, since I guess the water is treated?

Debes Christiansen: Even though we have treatment of water source then during winter storms sea water might contaminate inlead water.

DETECTION OF HPR0 IN DENMARK AND CRITERIA FOR DIAGNOSIS OF ISA

Helle Frank Skall
National Veterinary Institute, Technical University of Denmark

Abstract:

In August 2010 a surveillance sample from Atlantic salmon sampled in the broodfish section of a Danish farm producing salmon for restocking was tested positive for ISAV by RT-PCR. The fish were offspring of wild salmon and reared from eggs in a closed unit where after they were moved to an open unit containing wild fish (salmon and perch).
The samples consisted of kidney, heart and gills in RNA*later*. They were examined by conventional RT-PCR using the TK primers and by real time RT-PCR using the Gilad primers and 1/9 samples were positive. Sequencing of the HPR region identified the isolate as HPR0.
Samples from Atlantic salmon in the closed unit and from perch in the open unit were examined for ISAV with negative results.
No signs of disease consistent with ISA were observed at the facility.
How should this finding be interpreted?
In the OIE Diagnostic Manual and in the EU Commission Decision 2003/466/EC a number of findings are listed as causing ISA suspicion:

If only RT-PCR positive is this a suspicion?
Another set of criteria is stated for confirmation of ISA:

OIE	EU
Disease or pathological changes consistent with ISA	Disease or pathological changes consistent with ISA
Cell culture positive	Cell culture positive
Evidence for the presence of ISAV from two independent laboratory tests (RT-PCR and IFAT)	Evidence for the presence of ISAV from two independent laboratory tests (RT-PCR and IFAT)
Detection of antibodies to ISAV	Transfer of ISA-infected live fish to farm
	Epidemiological links

Comparison of
OIE (diagnostic manual) and EU (CD 2003/466/EC)

OIE (diagnostic manual)	EU (CD 2003/466/EC)
***Confirmation of ISA*:**	***Confirmation of ISA*:**
Disease **AND** IFAT on tissue preparations	Disease
AND	**AND**
Cell cultivation **OR** RT-PCR	Cell cultivation **OR** RT-PCR **OR** IFAT on tissue preparations
Confirmation of ISAV infection:	**OR**
Cell cultivation from two independent samples	Cell cultivation from two independent samples
AND (OR?)	**OR**
Cell cultivation from one sample **AND** RT-PCR or IFAT on tissue preparations	Cell cultivation from one sample **AND** RT-PCR or IFAT on tissue preparations
	Confirmation of ISAV infection:
	Not described

According to this set of criteria the finding did not lead to confirmation of neither ISA nor ISAV infection as positive cell cultivation is mandatory.

Furthermore, as the isolate was sequenced as HPR0, which has never been detected in ISA diseased fish, the Danish Competent Authorities conclusion was that Denmark is still free of ISA.

Minutes:

HPR0 was detected in Danish salmon produced for restocking purpose. In the OIE – manual of Diagnostic tests for Aquatic animals ISA is in one place defined as infection with salmon anaemia virus, but later the presence of disease is a key point. The Danish Competent Authorities concluded that Denmark is still free of ISA but it is clear that better guidelines are needed.

Questions:

Olga Haenen: Interesting thoughts. I will bring them to OIE. As things are now I think you should notify when you find HPR0.

Brit Hjeltnes: We in Norway are also pressing to get these issues raised.

Einar Karlsen: The problem with the OIE manual is that they do not discriminate between infection and disease but for HPR0 a fish can be infected but the virus will not induce disease.

Søren Kahns: Some of us are meeting later today to discuss this issue in more depth. Participants interested in this topic are welcome to join the discussion.

PD-VACCINE IS EFFECTIVE IN REDUCING SEVERITY OF DISEASE OUTBREAKS IN NORWEGIAN AQUACULTURE

B. Bang Jensen*[1], A.B. Kristoffersen[1], C. Myr[2] and E. Brun[1]
[1]*Norwegian Veterinary Institute, Oslo, Norway*
[2]*PD-fri/Norwegian Seafood Federation, Bergen, Norway*

Abstract:

Pancreas disease (PD) was first discovered in Norway in the mid-eighties. Until 2003, the yearly number of outbreaks was less than twenty, but in 2004 the number of outbreaks doubled, and in 2008, it was more than five times that of 2003. Since the disease is associated with massive losses in salmonid aquaculture, this increase in number of yearly outbreaks led to PD being controlled by national legislation from 2008. Furthermore, the aquaculture industry initiated a parallel project with the aim to reduce the consequences associated with PD (The PD-fri project). Mitigation measures where establishing of a PD-free zone, mandatory vaccinations outside the free zone and movement restrictions outside of and into the free zone. Also, the PD-fri project collated data on management, production and disease for individual seasites outside the PD-free zone.
Data from 201 cohorts of fish from seatransfer to slaughter was collected, from outset from spring 2007 to spring 2009. The data was then analysed in order to investigate the following risk factors: vaccination, strain of fish, other diseases and number of treatments against sea-lice. The effect was analysed with regards to length of PD-outbreaks, cumulative mortality, average weight gain and feed conversion ratio.
Univariat analyses revealed that there was significantly less PD-outbreaks and lower cumulative mortality among fish in vaccinated locations than non-vaccinated locations. Furthermore, the length of outbreaks was significantly shorter in locations with vaccinated fish than locations without.
Multivariate analyses using the probability of getting PD resulted in a model where vaccination and outbreak of Infectious Pancreas Necrosis were significant.
The effect of vaccination against PD has previously been regarded as poor, because it does not seem to reduce the number of outbreaks. This study shows that there are beneficial effects of PD-vaccination, especially in reducing mortality and length of outbreaks. The project would not have been possible without the involvement of the industry, both with regards to containing disease and to obtain data.

Minutes:

PDfri project was initiated to stop further spread of PD, reduce number of outbreaks, and reduce losses. 337 out of 342 sites agreed to participate and pay costs around 1250 euro. Norway was during the project divided into an endemic- and a non-endemic zone. The project showed good results and the fact that this was an industry-driven project meant that the involvement from the industry was high. PDfri has recommended surveillance of all locations in the endemic zone in order to detect infections as early as possible.

Questions:

No question.

EXTENSION OF EPIZONE

Management, control and surveillance of viral encephalopathy and retinopathy in aquaculture

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Abstract:

As part of the research project EPIZONE , a workshop on: " Management , control and surveillance of viral encephalopathy and retinopathy in aquaculture" will be organized during the next EAFP Conference. In order to obtain as much as possible information concerning the impact of the disease, the control measures applied and the results obtained, at global level, a questionnaire has been issued and sent to 26 nodavirus fish pathology experts.
On May, 15 seventeen documents, mainly referring to the Mediterranean region, were returned. Here only data concerning the situation in the Mediterranean area, provided by thirteen experts are presented and discussed. The information obtained confirms that viral encephalopathy and retinopathy (VER), is widespread in the Mediterranean Countries, causing significant economic losses except in Croatia, Turkey and France. In this last Country, in particular, the last VER outbreak dates back to ten years ago. Among all the Mediterranean Countries VER is notifiable only in Israel. In addition to this Country, Croatia also provides trade restrictions, particularly concerning imports from countries outside EU. With regard to bio-security measures, with rare exceptions, their application in hatcheries does not seem particularly strict and effective. The continuous introductions in the hatchery of wild broodfish, without a proper quarantine period and diagnostic screening to avoid potential carrier, represent a high risk of infection as well as the feeding with raw molluscs and fish.
European sea bass (*Dicentrarchus labrax*) is by far the most affected species, but there are other susceptible species such as meagre (*Argyrosomus regius*), Senegalese sole (*Solea senegalensis*), shi drum (*Umbrina cirrosa*), white grouper (*Epinephelus aeneus*), grey mullet (*Mugil cephalus*) and gilthead sea bream (*Sparus aurata*). This last species, which for many years has been considered completely resistant, has suffered, in recent years, serious mortalities even if limited to larval and juvenile stages. The prevalence of the disease varies widely from region to region, showing maximum values above 50%. Morbidity and mortality are age dependent, larvae and juveniles showing the most important losses. In sea bass, however, mortalities ranging between 25 and 50% in on-growing facilities are not uncommon.

Minutes:

In April 2011 a questionnaire was sent out to 26 experts on Nodavirus disease. 17 answers were received with requested information. The 13 answers from laboratories in the Mediterranean area are in focus in this presentation. The disease is only notifiable in Israel. Only 2 countries have an official surveillance plan in force. 8 experts regard VER/VNN as a significant problem for mariculture in their country. 5 experts would like the disease listed on EU level. 8 experts would like the disease listed on OIE level. Losses due to the disease range from below 10% to above 50%.

Questions:
No questions

SEROLOGICAL TESTS FOR SPECIFIC ANTIBODY DETECTION IN EUROPEAN SEA BASS (*D. LABRAX*) AGAINST VERV

Niccolò Vendramin and Elisabetta Cappellozza
IZSVe, Legnaro, Italy, ***E mail:*** *nvendramin@izsvenezie.it*

Abstract:

Viral encephalopathy and retinopathy is a serious disease causing significant economic damages to marine aquaculture industry. This disease has a wide geographical distribution as it has been observed in tropical and temperate climates. More than forty species, mainly of marine origin, have been affected so far and this number is likely to rise in future following the introduction of new species and the increase of aquaculture trade.

According to OIE, all fish mortalities characterized by abnormal swimming behaviours associated with vacuolar lesions in the nervous tissues containing viral particles of the *Nodaviridae* family should be ascribed to one single disease, officially identified as viral encephalopathy and retinopathy (VER), also known as viral nervous necrosis (VNN).

The presence of clinical symptoms depends on several factors such as species, age and temperature; furthermore, acute and sub-acute forms are characterized by different symptoms and mortality rates. The most characteristic and common clinical sign observed in the different species is an abnormal swimming behaviour characterized by a difficulty to maintain the normal static and dynamic equilibrium, speed and swimming direction and to control the swim bladder inflation.

In the Mediterranean Sea the disease appeared in the '90's, nowadays it is considered as an endemic disease and represents one of the major constraint of fish culture all over the world.

European Seabass (*D. labrax*) demonstrated to be one of the most susceptible species to VER in Mediterranean Sea, particularly in larval and juveniles stages. Adult fish may harbour the virus latently and spread the infection to the juveniles present in the farm.The disease proves to be a serious threat also for other species, such as Sea bream (*S. aurata*, particularly larval stage), Sole (*S. solea, S. senegalensis*) and Shi drum (*U. cirrosa*). Diagnostic techniques which have reached high levels of specificity and sensitivity have always aimed at the detection of aetiological agents with both classical viral and biomolecular techniques. Unfortunately these could only partially be applied to non-invasive samples. Few papers report of serological techniques used for the detection of specific antibodies in Sea bass. In this work three different serological techniques are described and compared: ELISA, Serum neutralisation and IFAT. Clinical data, obtained from the field, report of infection and mortality year after year of the same batch of fish reared in particular conditions. Serological data obtained with these tests seem to confirm the suspicion that very low immunitary memory characterise the batch of fish at different time points after the outbreak.

Minutes:

Comparison of three serological tests (ELISA, SNT, and IFAT) for detection of VER. Generally good accordance between different techniques in terms of positive/negative.

Questions:

Athanasios Prapas: You sampled in December and were still able to see clinical signs?

Niccolo Vendramin: Yes.

Athanasios Prapas: In Greece we find that infected fish are very resistant the year after.

Niccolo Vendramin: We find that outbreaks can occur again the year after.

SESSION IV: Update from the EURL

Chair*: Niels Jørgen Olesen*
Minutes: *Helle Frank Skall*

Norway is at present evaluating their surveillance system. In connection to this evaluation Britt Bang Jensen asked the audience about help to filling out a questionnaire regarding specificity and sensitivity of VHSV tests.

Niels Jørgen Olesen: Two of our colleagues will retire this year. One is Jeannette Castric, who unfortunately is not able to be on this meeting. She has been a tremendous help over the years.
The other is the young man, Giuseppe Bovo, who wants to settle in a small house in Sicily. Giuseppe has also done a tremendous work in this field in many years. Let us all applause these two persons and wish them good luck in the future.
In the afternoon we will have a little meeting at the institute at 2 o'clock discussing ISA HPR0 and you are all invited to participate.

I hope you all use the EURL website (www.eurl-fish.eu) where you can find a lot of information.
One of our duties as the EURL is to harmonize diagnosis in the NRL's and the proficiency test are part of that. We also supply a number of standard sera and reagents to other laboratories. We try to produce reagents to be used in various laboratories. Last year we produced antisera against KHV. It is also important to keep and maintain a library of isolates and we are grateful for each isolate provided by you to be included in this library.
We also assist member states on diagnosis and characterization of diseases and isolates, and we are very happy for the collaboration that we have with you all in this respect.
The fish pathogens database now contains both VHSV and IHNV and we hope the database will include more diseases in the future.
We collect information regarding the disease information and production in Europe through S&D. All information can be found at the web page and we encourage you to look at it, and please inform us, if some information should be added or if you do not agree in the data.
We also provide training both through courses at our laboratory and at missions to different laboratories.
We have been working a long time on VHSV qPCR and we are on the steps to send in a manuscript on this subject so the method can be included in the manuals.

WORK PROGRAMME FOR THE EUROPEAN UNION REFERENCE LABORATORY FOR FISH DISEASE, 2010

Søren Kahns, Nicole Nicolajsen, Søren Peter Jonstrup and **Niels Jørgen Olesen**

Every year a work programme of the EURL for the following year is submitted to the Commission for approval. The programme for 2010 was as follows:

I. LEGAL FUNCTIONS AND DUTIES

The functions and duties of the Community Reference Laboratory are described in the Council Directive 2006/88/EF Annex VI part I

II. OBJECTIVES FOR THE PERIOD JANUARY - DECEMBER 2010

1. Organise and prepare for the 14th Annual Meeting for the National Reference Laboratories for Fish Diseases in 2010.
2. Produce a report from the Annual Meeting 2010.
3. Collect 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
4. Identify and characterise selected isolates of listed viruses (serological and genetic characterisation)
5. Production of antisera against selected isolates when necessary.
6. Assessment and standardisation of Real-time PCR tests for the diagnosis, identification and typing of the listed non-exotic fish diseases.
7. Expanding www.fishpathogens.eu with IHNV, SVCV and the inclusion af KHV and ISA will be initiated.
8. 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).
9. Update the webpage for the CRL, www.crl-fish.eu
10. Update and include standard operating procedures on the CRL web page for the listed exotic and non-exotic diseases
11. Workshop on available kits and reagents for diagnosis of the listed non-exotic diseases VHS, IHN, ISA and KHV including consideration of their sensitivity and specificity.
12. Supply standard antisera and other reference reagents to the National Reference Laboratories in Member States.
13. Inclusion of SOP´s on serological methods for detection of fish antibodies against VHSV, IHNV and KHV on CRL website, and introducing the methods in new Commission Decision on sampling and diagnostic procedures
14. Prepare the Annual Inter-laboratory Proficiency Test year 2010 for the National Reference Laboratories. The test will be expanded to also include ISAV and KHV.
15. Collate and analyse information gained from the Inter-laboratory Proficiency Test
16. Establish diagnostic methods for diagnosis of EUS and assess the possibilities for including *Aphanomycis invadans* in proficiency test in future.
17. Facilitate and provide training in laboratory diagnosis.
18. Attending missions, international meetings and conferences. Missions will focus on NRLs where on-site communication would be beneficial. And to reference laboratories on listed exotic and non-exotic fish diseases in order to be updated on diagnostic methods.

WORK PROGRAMME FOR THE EUROPEAN UNION REFERENCE LABORATORY FOR FISH DISEASE, 2011

Niels Jørgen Olesen, Nicole Nicolajsen, Søren Peter Jonstrup, Maj-Britt Christophersen and Søren Kahns

The work plan for the current year is as follows:

OBJECTIVES FOR THE PERIOD JANUARY - DECEMBER 2010

The functions and duties of the European Union Reference Laboratory for Fish Diseases (EURL) are described in the Council Directive 2006//EF Annex VI part I 88

OBJECTIVES FOR THE PERIOD JANUARY - DECEMBER 2011

1. Organise and prepare for the 15th Annual Meeting for the National Reference Laboratories for Fish Diseases (NRLs) in 2011.
2. Produce a report from the Annual Meeting 2011.
3. Prepare the Annual Inter-laboratory Proficiency Test year 2011 for the NRLs. The test will include VHSV, IHNV, EHNV, ISAV and KHV.
4. Collate and analyse information gained from the Inter-laboratory Proficiency Test
5. Supply reference reagents to the NRLs in Member States.
6. Production of antisera against selected isolates when necessary.
7. 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*.
8. Establish and maintain a library of tissue material from fish infected with listed pathogens.
9. Update the webpage for the EURL, www.crl-fish.eu (the web address will be changed to www.eurl-fish.eu in autumn 2010)
10. Update the diagnostic manuals for VHS, IHN, ISA, KHV disease and EHN on the EURL web page, and include diagnostic manuals for EUS.
11. Update diagnostic methods for diagnosis of Epizootic Ulcerative Syndrome (EUS) and assess the possibilities for including *Aphanomyces invadans* in proficiency test in the future.
12. 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.
13. Identify and characterise selected isolates of listed viruses (serological and genetic characterisation).
14. Update and expand www.fishpathogens.eu with more pathogens.
15. Perform molecular epidemiology analysis to improve knowledge on diseases spreading mechanisms of viral pathogens
16. Assessment and standardisation of real-time PCR tests for the diagnosis, identification and typing of the listed non-exotic fish diseases.
17. Facilitate and provide training in laboratory diagnosis. A training courses in methods used for diagnosis of fish diseases will be established and 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.
18. Organizing missions to relevant laboratories. Missions will focus on NRLs where on-site communication would be beneficial.
19. Organise a workshop on “Surveillance and Epidemiology of Diseases in Aquaculture” at the premises of the EURL-Fish and in collaboration with the OIE collaborating centre for aquatic epidemiology and the EURL-Molluscs and –Crustacians, respectively.
20. Attending missions, international meetings and conferences in order to be updated on diagnostic methods on listed exotic and non-exotic fish diseases.

OBJECTIVES FOR THE PERIOD JANUARY - DECEMBER 2012

1. Organise and prepare for the 16th Annual Meeting for the National Reference Laboratories for Fish Diseases in 2012.
2. Produce a report from the Annual Meeting 2012.
3. Collect 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.
4. Identify and characterize selected isolates of listed viruses (serological and genetic characterization).
5. Production of antisera against selected isolates when necessary.
6. Assessment and standardization of Real-time PCR tests for the diagnosis, identification and typing of the listed fish diseases.
7. Update and expand www.fishpathogens.eu with other pathogens.
8. 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).
9. Update the webpage for the EURL, www.eurl-fish.eu
10. Establish and maintain a library of tissue material from infected fish.
11. Update the diagnostic manuals for the listed diseases on the EURL web page.
12. Supply standard antisera and other reference reagents to the National Reference Laboratories in Member States.
13. Perform molecular epidemiology analysis to improve knowledge on diseases spreading mechanisms of viral pathogens
14. Prepare the Annual Inter-laboratory Proficiency Tests year 2012 for the National Reference Laboratories. The tests will include VHSV, IHNV, EHNV, ISAV, KHV and *Aphanomyces invadans*.
15. Collate and analyse information gained from the Inter-laboratory Proficiency Test
16. Facilitate and provide training in laboratory diagnosis.
17. Offering the yearly training courses in methods used for diagnosis of fish diseases, at the EURL laboratory facilities. The content will depend on request from participants
18. Organizing missions to relevant laboratories. Missions will focus on NRLs where on-site communication would be beneficial.
19. Attending missions, international meetings and conferences in order to be updated on diagnostic methods on listed exotic and non-exotic fish diseases.
20. Any other suggestions?

Minutes:

Apart from all the mandatory tasks for next year, suggestions for other topics to the workprogramme ofthe EURL would be most appreciated

The backbone of our work program for 2011 is similar to our work program for 2010. However extensions have been made. A new thing is to establish and maintain a library of tissue material from fish infected with VHSV, IHNV and IPNV that can be sent to the laboratories for use in validation of methods.

The proficiency test for 2011 will most likely include EUS as well as the viruses included last year. The test will be sent out in October. The test will then comprise all the listed disease pathogens. For EUS we will send out inactivated material to be sure we do not spread the disease! For those of you who need positive control material please contact us. I am sure Birgit Oidtmann will also be helpful providing positive control material.

Regarding diagnostic manuals we have already uploaded manuals for VHS, IHN and EHN. The ISA manual was uploaded but only diagnostic procedures are included not the sampling procedures. The diagnostic manual for KHV will be uploaded soon.

For EUS we have used significant resources on updating and implementing diagnostic procedures.

We have also done a lot of work on epidemiology on VHS.

Later this year we will organize a workshop on Surveillance and Epidemiology of Diseases in Aquaculture. We will host the workshop, but the organization of the workshop is being done in collaboration with the newly appointed OIE collaborating center on aquatic animal epidemiology. You will find an invitation in the folder. The WS will primarily be for colleagues who already have some knowledge on epidemiology. The Commission will invite 1 person from each member state who will have travel and living paid for. But the workshop will be open for all interested and there will be space for app. 60 participants.

We would like to ask you if some of you would like us to make a EURL visit to your laboratory in 2011? Such visit or mission would provide a possibility for exchanging know-how and obtaining experiences in your own laboratory premises in specified diagnostic procedures. We have already talked with the Spanish NRL about a visit to their laboratories but if others are interested please contact us.

In 2012 we will again organize a training course. The course will be advertised in November 2011 and we encourage you to inform us on which topics you will like us to include in the course.

Suggestions for the workplan 2012:
Provide recommendations for strain discrimination of listed pathogens e.g. marine VHS/highly pathogenic VHS strains; ISA HPR0; ranavirus/EHNV; KHV variant strains.
More focus could be added on “emerging” non listed diseases like nodavirus, aquatic alphaviruses. It is a requirement in the legislation to be aware of emerging diseases.
To extend the FishPathogens database by including more pathogens. We hope in 2011 to include nodavirus through an extension of the Epizone project.

If the laboratories have suggestions for tasks to include in the workplan for 2012 please contact us. The workplan has to be sent to the commission by 1 September 2011.

Are there any comments/suggestions?

Olga Haenen: First I would like to congratulate you for a very successful meeting once again. Would you also consider topics beyond virology like e.g. bacteriology.

Niels Jørgen Olesen: We have focused mainly on the listed diseases as this is our task.

Brit Hjeltnes: You have of course to focus on listed diseases, but you also have to be vigilant on the emerging diseases and I think this could be included in the next meeting. We would then have to concentrate on the really important emerging disease like e.g. PD.

Britt Bang Jensen: In the new regulation it is stated that you should focus on increased mortality, how is this handled in the different member states, at what criteria do we recognize it as increased mortality – this is a subject which could be included in next year's meeting.

EURL TRAINING COURSE 2011 AND REQUEST FOR IDEAS FOR 2012

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Abstract:

From late January to early February 23 participants mainly from European NRLs for Fish Diseases participated in an EURL training course in Aarhus, Denmark. Participants could choose from one to three modules each lasting three days. One module focused on PCR, one on cell cultures, and one on Immunochemical methods. Here I present a small update from this course and afterwards we will ask you for ideas for next year's course. So please have your ideas ready.

Minutes:

I will tell you about the training course we had this year and the plans for the coming training course.

We had very good feedback from the participants at the first course. We plan for a course to take place next year and we encourage participants to provide topics for training.

Questions

Olga Haenen: What about double and triple viral infections?

Niels Jørgen Olesen: I think this could be a good idea.

RESULTS AND OUTCOME OF PROFICIENCY TEST, PT1, 2010

Søren Kahns, Nicole Nicolajsen, Maj-Britt Christophersen and Niels Jørgen Olesen
EU Reference Laboratory for Fish Diseases, Technical University of Denmark, Aarhus, Denmark

Abstract:
A comparative test of diagnostic procedures was provided by the EU Reference Laboratory (EURL) for Fish Diseases to 38 National Reference Laboratories (NRLs) in the start of September 2010. The test was prepared and tested according to protocols accredited under DS/EN ISO/IEC 17025 and ILAC-G13:08/2007 standards. The test contained five coded ampoules with the following content VHSV genotype Ia, IHNV genogroup M, EHNV, European catfish virus (ECV), and spring viraemia of carp virus (SVCV), The proficiency test was designed to primarily assess the ability of participating laboratories to identify the notifiable fish viruses VHSV, IHNV and ENHV (all listed in Council Directive 2006/88/EC).
Participants were asked to titrate the viruses to assess the cell susceptibility for virus infection in the respective laboratories. 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 monolayered cell cultures followed by e.g. neutralisation test, immunofluorescence, ELISA or PCR. If ranaviruses should be present in any of the ampoules, it was mandatory to perform a sequence analysis of the isolate in order to determine if the isolate is EHNV.

Outcome of PT1
18 laboratories out of 38 correctly identified all viruses in all ampoules correctly and obtained maximum score. 10 laboratories did not identify the virus in ampoule III as ECV, ESV or rana but not EHNV. Nine laboratories did not identify virus in one or more ampoules where a virus was present. 5 laboratories observed additional virus than present in the ampoule. 25 laboratories used ELISA for identification of viruses. 23 laboratories used IFAT for identification of viruses. 8 laboratories used neutralisation tests for identification of viruses. 35 laboratories used PCR for identification of viruses. 30 laboratories performed sequencing for identification of viruses. 32 laboratories used BF-2 cells. 34 laboratories used EPC cells. 14 laboratories used RTG-2 cells. 16 laboratories used FHM cells

Concluding remarks
EHNV was included and so was the ECV that belongs to the ranavirus family. All the 24 laboratories performing sequencing of the isolate in ampoule I identified the virus correctly as being EHNV. 24 laboratories identified the virus in ampoule III as ranavirus but not EHNV. However, 7 other laboratories that performed sequencing of the ECV isolate in ampoule III identified the virus as EHNV although the submitted sequences in 6 cases were identical to ECV/ESV. We recommend that laboratories carefully analyse their sequencing results when a ranavirus is identified in order to rule out if the virus is the listed EHNV or not. Furthermore,

The EHNV was present in a relative low titre, which likely is the reason why 8 laboratories did not identify any virus in this ampoule. We can only recommend that participant subcultivate the samples as it is described in the Commission Decision 2001/183/EC as subcultivation increases the possibilities of isolating low titre viruses.

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

RESULTS AND OUTCOME OF PROFICIENCY TEST, PT2, 2010

Søren Kahns, Nicole Nicolajsen, Maj-Britt Christophersen and Niels Jørgen Olesen
EU Reference Laboratory for Fish Diseases, Technical University of Denmark, Aarhus, Denmark

Abstract:
It was decided at the 14th Annual Meeting of the NRLs for Fish Diseases in Aarhus 26-28 May 2010, that testing for ISAV and KHV (both listed in Council Directive 2006/88/EC) for the first time should be included in the yearly proficiency test provided by the EURL. Therefore, a comparative test, PT2, of diagnostic procedures was provided by the EU Reference Laboratory (EURL) for Fish Diseases to 36 National Reference Laboratories (NRLs) in the start of September 2010. The test contained five coded ampoules of which two contained ISAV, two contained KHV and one did not contain any virus.

Participants were expected to use their normal PCR or real-time PCR methods for detection of KHV and their normal RT-PCR or real-time RT-PCR methods for detection of ISAV. It was not mandatory to grow KHV and ISAV on cell cultures in this test, but the viruses has not been inactivated and should thus be viable and possible to amplify in cell cultures.

Outcome of PT2
23 laboratories out of 36 correctly identified all viruses in all ampoules and obtained maximum score. Two laboratories only examined for KHV and not ISAV and one laboratory only examined for ISAV and not KHV. Of these three laboratories, two laboratories presented correct answers and obtained the score of 6 out of six. Laboratories scoring 8, 6 or 4 either lacked virus identification or identified additional viruses than those present. Two laboratories did not submit any results and obtained the score of 0.

12 laboratories used ISAV real-time RT-PCR. 24 laboratories used ISAV RT-PCR. 4 laboratories used both ISAV real-time RT-PCR and ISAV RT-PCR. 14 laboratories used KHV real-time RT-PCR. 30 laboratories used KHV PCR. 11 laboratories used both KHV real-time PCR and KHV PCR

Concluding remarks
Considering that this was the first time that the EURL provided a proficiency test on ISAV and KHV identification, we think that most participants obtained satisfying results. All 33 laboratories performing KHV identification did correctly identify KHV in the ampoule containing high titre KHV. All 31 laboratories performing ISAV identification, except two, did correctly identify ISAV in the ampoule containing high titered ISAV. Lowering the titre of the virus caused a few laboratories to miss identification of KHV and ISAV in the low titered ampoules. To decrease the risk of having false negative results, it is always recommended that laboratories use the most sensitive tool available, validate the sensitivity of their diagnostic tools and use proper controls.

One ampoule containing no virus was included in the test and 34 out of 31 laboratories correctly identified that the ampoule was negative for virus. Three laboratories identified KHV in this ampoule, probably due to cross contamination problems. False positive results is a common critical problem in PCR based diagnostics as PCR or RT-PCR can detect very few copies of DNA or RNA, respectively. Therefore, it is extremely important to minimise the risk of cross contaminations.

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

Minutes:

Together with the proficiency test we sent out a questionnaire regarding the accreditation status in the different laboratories both regarding exotic and non exotic diseases.
Many laboratories are accredited for performing cell culture assays for VHS and IHN. Fewer laboratories are accredited for PCR methods that are the main tools for diagnosing KHV and ISA. For EHN some are accredited and only few for EUS. I want to stress that these are results from all participants in the proficiency test and not just the EU member states.

Regarding the proficiency test all EU member states participated or had representatives.
Most parcels were delivered within 3 days after sending. Some were delivered later due to reasons out of our hands. Loggers were included in many of the parcels.

The proficiency tests were prepared according to protocols accredited according to ISO 17025 and ILAG guidelines.

The samples were lyophilized before sending out. We generally see a decrease in titer after lyophilization but all titers were above detection level.

It is important that the laboratories both follow the procedures laid down in the EC regulations but also that the normal procedures in the laboratory as this is a test on the work normally done in the laboratory.

In case of no CPE it is also important to subcultivate the samples as the titer may be low.

PT1
Most laboratories did very well in PT1 but a few laboratories can improve.

If your cell line is performing below the 25% quartile in the titration you should consider if you may need to replace your cell line. If this is the case you are welcome to contact us.

Regarding test methods used by the participants, ELISA and IFAT is used a lot among the participants as seen in the earlier years. But this year PCR and sequencing has been used among a lot of the laboratories also.

Most laboratories used sequencing to discriminate between the ranaviruses and one lab used RFLP.

Generally laboratories performed well on PT1 but there were a few problems with labs not analyzing sequencing results of the ranaviruses properly and thus not being able to tell whether it was EHNV or not. Very few laboratories have not implemented PCR yet, which is a requisite for being able to diagnose all the listed diseases.

PT2
This year KHV and ISA were included in the proficiency test. Each of the isolates was send out in a high titer and a lower titer version and we also sent a blank sample.
Most laboratories were able to identify the virus but a few labs were not able to identify the virus with low titers and a few labs had contaminated the blanks. A few laboratories only examined for one of the viruses.

Regarding the genotyping we will be more specific on which genotyping system we will like you to use in the next proficiency test as the laboratories used different genotyping systems.

Feedback on possible improvements

In general the feedback was very positive, only "critical" comments were shown. Some laboratories would like more time to solve the test and we will try to see if this will be possible this year.
Laboratories were complaining that the glass ampoules are difficult to open. Unfortunately we will not be able to use the rubber ampoules as the titers do not keep well in these.

Questions

Olga Haenen: A remark on ampoule 1. We tried to inoculate on F25 flask and the sample was negative still after 3 passages. On 6 well plates we saw one plaque at the end of the second passage. It seemed that we generally had better growth on 6 well plates than on flasks. The pH seemed to be o.k. in both systems, concerning the color of the medium. Did anyone of you have similar problems? We now always use as well 6 well plates as small flasks, not to miss any cpe.

Sigrid Cabot: I want to congratulate you all for the very good results. Secondly I will like to thank Niels for a very excellent meeting. Regarding priorities the listed diseases are of course the top priority but also the emerging diseases are an important issue.
I would also like to thank Giuseppe for his excellent work and contribution to the work of the Commission through his participation in numerous expert- and ad hoc groups.

CLOSING REMARKS

Where and when should we have our next meeting? Last year we were talking about organizing the meeting in another laboratory as it is nice to visit the other laboratories. Unfortunately it is not allowed to organize the meeting in other countries unless there are very good scientific reasons for this.

Brit Hjeltnes: It is difficult to reach a decision when and where. You should have a look on when and where it is practical. For next year meeting, one could consider including topics that are relevant for the Industry.

Niels Jørgen Olesen: One idea could be to visit the brand new facilities of FLI.

Sigrid Cabot: I cannot promise you it will be possible to go anywhere else than Denmark but if you have reasons to go elsewhere you might apply and we will have a look at it.

Niels Jørgen Olesen: If you know of meetings etc that would coincide with this meeting please tell us these dates so we do not organize overlapping meetings. I will like to thank both the audience and the speakers for participating and thanks to all for having provided abstracts for the booklet. I will like to ask the speakers if it will be OK for us to upload your presentations. Please contact Søren Kahns or Nicole Nicolajsen.
I will also like to thank Sigrid for helping us. And big thanks go to Nicole Nicolajsen for all her work in organizing this meeting and also thanks to Søren Kahns.
Torsten Boutrup, Søren Peter Jonstrup and Helle Frank Skall have been taking the minutes. A report of the meeting will be made and send to all of you.

Pictures

Guiseppe Bovo, Olga Haenen, Vlasta Jencic and Nicole Nicolajsen were excellent photographers during the workshop. For pictures from the Annual Meeting please have a look at our web page. http://www.eurl-fish.eu.