Report on the 13th Annual Meeting of the National Reference Laboratories for Fish Diseases

Copenhagen, Denmark
May 26-28, 2009

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

Contents

Introduction and short summary

In 26-28 May 2009 the 13th annual meeting of the National Reference Laboratories for fish diseases was held back-to-back with a mini-workshop on implementation of Council Directive 2006/88/EC. A total of 61 participants from 35 countries attended over the three day period. There were five sessions with a total of 32 presentations, 7 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 presented new findings from their home countries. Scotland UK had experienced outbreak of ISA and presented the investigation done into this. Subsequently, a study on tracing of spread of ISAV in Norway was presented, followed by a talk on the general disease situation in Norway. Later in this session presentations about viral fish diseases from Poland, IPN eradication in Switzerland, isolation of iridovirus from Hungary, a worldwide questionnaire on KHV organised within the EPIZONE project, and KHV epidemiology in Germany were given.

The session on technical issues related to sampling and diagnosis were divided into two parts. The first session focussed on diagnostic PCR setup. Here we were informed about the results of the 2008 KHV ring trial, how controls can be used in PCR analyses, application of a new assay for KHV detection and how a real-time PCR can be applied for routine detection of fish pathogens.
The last part of this section focussed on antibody based diagnosis and presentations were given on the ring trial for detection of VHSV and IHNV antibodies, ELISA tests for detection of CyHV-3 antibodies and serological methods for detection of KHV.

A mini-workshop on the implementation of Council Directive 2006/88/EC was held right after session two of the Annual Meeting. The workshop started with four presentations dealing with key issues to be addressed when implementing the Directive, on sampling and diagnostic plans for the listed diseases, on how to risk rank fish farms and finally on how fish farms have been categorised in EU according to the answers given in the Annual S&D questionnaire. Subsequently, participants were divided into three groups: 1) participants from Northern Europe, 2) participants from Continental Europe and 3) participants from Mediterranean European countries. Within these groups, participants from each country presented how the implementation process had progressed in their country. At the end of the mini-workshop all participants were gathered together and a summary from the three group discussions were given.

At the evening after the mini-workshop, the participants were invited to a banquet dinner at the Restaurant “Bastionen og løven”, located at one of the old fortifications in Copenhagen.

The last day was opened by an update session on scientific research. At this session, presentations were given on: 1) susceptible fish species to the listed diseases, 2) the database for fish pathogens, 3) molecular characterisation of VHSV and IHNV in Germany, 4) development of genotype specific monoclonal antibodies against VHSV and 5) status of the RANA-project.

The annual meeting ended with the traditional update from the CRL. The results of the proficiency test 2008 were presented. A report from a year with focus on training of laboratories and the thoughts and considerations about implementing the new listed diseases in our work was given. Furthermore, proposals on the CRL work plans for 2010 were discussed.

Minutes from the meeting were taken by Helle Frank Skall, Søren Peter Jonstrup, Britt Bang Jensen and Søren Kahns, 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 presentations. 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 Søren Kahns, Niels Jørgen Olesen, Britt Bang Jensen, Helle Frank Skall and Nicole Nicolajsen, with the help from the rest of the fish disease section at DTU Vet.

The meeting next year is tentatively planned for May 25-27, 2010, but more details will follow.

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

Århus, 28 August 2009

Niels Jørgen Olesen and Søren Kahns

Programme

Tuesday May 26th – Annual Meeting of the National Reference Laboratories

REGISTRATION AND WELCOME ADDRESS

13:00 – 13:30 **Welcome Address and announcements**
Søren Kahns (Community Reference Laboratory)

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

Chair: *Fiona Geoghegan*

13:30 – 14:00 Overview of the disease situation in Europe – *Niels Jørgen Olesen*
14:00 – 14:30 Occurrence of Infectious Salmon Anaemia (ISA) in Scotland UK – *Rob Raynard*
14:30 – 14:50 Tracing the spread of Infectious Salmon Anaemia virus (ISAV) in salmon farms in Norway – *Trude Marie Lyngstad*
14:50 – 15:10 Fish health trends and developments in Norwegian aquaculture 2008 – *Hege Hellberg*

15:10 – 15:40 Coffee break

14:40 – 14:55 Monitoring of viral fish diseases in Poland in 2004-2008 – *Marek Matras*
14:55 – 15:10 Eradication of IPN in a farm with chronically infected stocks – *Thomas Wahli*
15:10 – 15:25 Total eradication of VHS in Denmark – *Henrik Korsholm*
15:25 – 16:45 Isolation of an Iridovirus (Ranavirus) from catfish *Ameiurus nebulosus* in Hungary – *Tamás Attila Juhász*
16:45 – 17:05 Koi Herpesvirus disease: Data from the global EPIZONE questionnaire 2009 – *Olga Haenen*
17:05 – 17:20 Epidemiology and combat against fish epidemics - thoughts on koi herpesvirus disease (KHVD) – *Sven Bergmann*

Wednesday May 27th – Annual Meeting of the National Reference Laboratories

SESSION II: Technical issues related to sampling and diagnosis

Chair: *Søren Kahns*

8:30 – 10:30 *The first part of this session will focus on diagnostic PCR set-up: Which PCR/real-time PCR to choose, sampling procedures, prevention of contamination, controls, number of replicates, etc*

- The 2008 KHV PCR methods ring trial - *Keith Way*
- Application of controls that minimises the risk of obtaining false-positive or false-negative results in diagnostic PCR assays - *Søren Kahns*
- Implementation of a one-tube assay for koi herpesvirus (KHV) detection adapted to latent infected carrier fish - *Sven Bergmann*
- Development of a robust accredited real-time PCR laboratory system and its application to the routine detection of fish pathogens - *Mike Snow*

Subsequently, a discussion in plenum will follow on how to do PCR diagnostic in "your" lab.

10:30 – 11:00 Coffee break

Chair: *Pia Vennerström*

11:00 – 12:30 The last part of this session will focus on antibody based diagnosis – with the following presentations:

- Results from an inter-laboratory proficiency test on detection of antibodies against VHSV and IHNV in rainbow trout – *Jeanette Castric*
- Optimisation and validation of an ELISA for detection of antibody to cyprinid herpesvirus 3 (CyHV-3, KHV) – *Keith Way*
- Development of serological methods for detection of Koi herpes virus (KHV) antibodies in carp, *Cyprinus carpio* – *Jeanette Castric*

12:30 – 13:30 Lunch

Wednesday May 27th – Mini-Workshop on implementation of Council Directive 2006/88/EC

Chair: *Niels Jørgen Olesen*

13:30 – 13:50 The Key issues to be addressed when implementing the Council Directive 2006/88/EC – *Sigrid Cabot*
13:50 – 14:10 Sampling and diagnostic plans on VHS, IHN, KHV and ISA – *Giuseppe Bovo*
14:10 – 14:40 A model for risk ranking fish farms to inform disease risk-based surveillance – *Birgit Oidtmann*
14:40 – 15:00 Summary of categorisation of Fish Farms in EU according to S&D questionnaire – *Niels Jørgen Olesen*

15:00 – 15:10 Coffee break (in groups)

15:10 – 16:30 In groups: discussion on authorization and categorization and risk management. Each NRL is requested to present how the directive has been implemented in their country – 3 groups

Group 1 Chair: *Birgit Oidtmann*
Northern European countries
Group 2 Chair: *Niels Jørgen Olesen*
Continental European countries
Group 3 Chair: *Giuseppe Bovo*
Mediterranean European countries

16:30 – 17:00 Summarisation of the group discussions

19:00 ***BANQUET DINNER***

Thursday May 28th – Annual Meeting of the National Reference Laboratories Continued

SESSION III Scientific research update

Chair: *Hege Hellberg*

9:00 – 9:20 Susceptible species to listed diseases – EFSA Report – *Ana Afonso*
9:20 – 9:40 Establishment of a CRL-database for fish pathogenic viruses – *Søren Peter Jonstrup*
9:40 –10:00 Molecular characterization of VHSV and IHNV in Germany – *Heike Schuetze*
10:00 – 10:20 Distinction between genotypes of Viral Haemorrhagic Septicaemia virus (VHSV) using Monoclonal Antibodies – *Takafumi Ito*
10:20 – 10:40 Status from the RANA-project – *Britt Bang Jensen*
10:40 – 11:00 Open for presentation from participants

11:00 – 11:30 Coffee break

SESSION IV: Update from the CRL

Chair: *Niels Jørgen Olesen*

11:30 – 11:50 Report from Year 2008 – *Niels Jørgen Olesen*
11:50 – 12:15 Workplan for 2009 and 2010 – *Niels Jørgen Olesen*
12:15 – 12:35 Inter-laboratory Proficiency Test 2008 – *Søren Kahns*
12:35 – 13:00 Next meeting and end of 13th Annual Meeting - *Niels Jørgen Olesen*

13.00 ***Sandwiches and goodbyes***

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

Overview of the disease situation in Europe

Niels Jørgen Olesen & Nicole Nicolajsen
CRL for Fish Diseases, National Veterinary Institute, Technical University of Denmark

Minutes

This presentation is a presentation we have had all the years, starting out with each participant presenting data from their own country/region – this took too much time and was actually a boring way of presenting the data. 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 CRL web site (www.crl-fish.eu), where all raw data can be obtained. The S&D have evolved over the years to now comprise 5 parts: General data on production, epidemiological data on diseases, laboratory data from NRLs and other laboratories, quality assurance in NRLs and regional laboratories, and as the final part we have included categorisation of fish farms according to the new legislation in EU. Categorisation will be discussed in detail tomorrow. 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 2007 has risen a bit again after a decrease from 2003-2006. Data from 2008 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.

In Northern European countries there are mainly salmonid farms, in continental Europe we find a lot of carp farms, and in the Mediterranean area, besides carps, seabream and seabass are also species that many produce. Turkey is a big producer of rainbow trout and lots of rainbow trout farms is found in this country.

Concerning the epidemiological data, what is the distribution and amount of infected fish farms in Europe? For the first time ever no farms are considered infected with VHS in Denmark since March 2009. There seems to be severe underreporting of VHS and IHN in many countries. The infection status is known for about ½ of the farms.

The figures for KHV only reports on carp farms and not outbreaks in private garden ponds. The infection status regarding KHV is unknown for many carp farms, whereas for farms producing Atlantic salmon, the infection status for ISA is known for nearly all farms. For ISA app. ½ of the farms are considered infected at the Faroe Islands, but HPR0 positives only. Unfortunately, a new outbreak of ISA was observed again at the Shetland Islands after a pause of several years, and ISA is still a problem in Norway.

Many countries have surveillance programmes for SVC, BKD, and IPN, for which they are seeking "additional guaranties". The number of farms in the programmes varies from very few farms to many farms. Fewer countries have surveillance programmes for *Gyrodactylus salaris*.

There is very large differences between countries on how many samples are tested on cell cultures, ranging from < 100 to several thousands. PCR is really starting to come up in many countries, but 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.

More and more laboratories are becoming accredited according to the ISO 17025 standards.

Questions

***Rob Raynard*:** In general, how can we tell that the disease situation is improving?

***Niels Jørgen Olesen*:** The number of category I farms have increased dramatically, compared to the number of farms in VHS and IHN-free zones or compartments under the previous legislation. The new legislation tries to push people to not stay in the unknown category III group. All NRL's could help pushing the veterinary authorities to obtain knowledge on the disease status in the farms in their respective countries.

Stig Mellergaard: Do you think that the SVC surveillance will die out, now it is not listed anymore?

Niels Jørgen Olesen: Yes, for those countries that does not wish to obtain additional guaranties.

Occurrence of Infectious Salmon Anaemia (ISA) in Scotland UK

Rob Raynard*, Charles Allan, Sandy Murray, Mike Snow, Eann Munro, David Bruno, David Smail
Marine Scotland, Marine Laboratory, 375 Victoria Rd, Aberdeen, AB11 9DB Scotland. r.raynard@marlab.ac.uk

Abstract: On the second of January 2009, Infectious Salmon Anaemia (ISA), a disease listed as non-exotic to the EU under Directive 2006/88/EC, was confirmed on an Atlantic salmon ongrowing sea water site in the Shetland Islands of Scotland. A further 2 sites in the same area were officially suspect for ISA due to having received fish from the confirmed site in June 2008.

In accordance with Article 53 of Directive 2006/88/EC the Shetland Islands were temporarily suspended from freedom for ISA based on epidemiological evidence.

This was the first occurrence of ISA in Scotland since the first outbreak in the period 1998-1999 which was successfully eradicated. Measures for containment and eradication were put in place including the establishment of a protection zone and surveillance zone. Of the 17 sites inspected and screened within the ISA containment area, 3 sites were confirmed ISA positive. The first site tested positive by qRT-PCR and virus isolation, the second site was confirmed positive by qRT-PCR, virus isolation and IHC on 30 January and a third site was confirmed by qRT-PCR and clinical and postmortem observations on 20 March. All of the fish held on the confirmed and suspect sites were withdrawn under official supervision.

The conclusion of investigations into the potential spread and origin of infection are that, that infection is restricted to the containment area in South West Shetland, within which it appears to have spread hydrodynamically. The source of infection is unknown. Since the entire UK had previously been free of ISA it is possible that ISA has emerged from a wild source or from import. Genetic analysis carried out on the HE gene of virus from the confirmed cases shows that the sequence is unique representing a novel HPR type. The virus is a member of group EU G1 and the sequence is not closely related to previous sequences identified in Scotland.

Surveillance will continue in the Containment area according to Decision 2003/466/EC. The Government's objective is to eradicate ISA from the area and to regain freedom from ISA for South West Shetland.

Minutes

First I will like to inform you, that there has be a reorganisation in Scotland affecting our laboratory, so we are now merged with the authorities and called Marine Scotland, we still carry out the same functions though.

ISAV is an Orthomyxovirus causing significant mortality in farms. ISA has occurred worldwide in Atlantic salmon aquaculture. ISAV is capable of mutation. Genetics suggest a different origin for European and North American isolates.
In May 1998 to May 1999, Scotland experienced 11 ISA cases, and has been free of ISA since 2004 until last year.
The Shetland Islands are situated halfway between Norway and the Faroe Islands, and they feel quite independent, also from Scotland.
In the first farm diagnosed with ISA, a large number of earlier mortalities were attributed to causes other than ISA. Abnormal behaviour was seen in some fish. During inspection, some signs of disease were observed, but not indicative of ISA. Diagnostic samples were taken. ISA was confirmed 2nd January 2009. Containment area, protection zone and surveillance zones are established. It is an important area as 10% of the national salmon production is produced there corresponding to approximately 13.000 tonnes.
Diagnosis: mainly clinical and post mortem, virus isolation, PCR, histopathology, but also IHC and IFAT. Evidence of circulatory disturbance and damage to endothelial cells, virus identification on TO cells using a haemadsorption test to detect intra-cellular virus. Positive cultures are confirmed by IFAT.
Clinical inspection at least monthly in the control zone, every 2 months in the surveillance zone, or the sites with epidemiological links, weekly reporting of mortalities, and targeted surveillance in the protection zone.
Until now we have 5 cases: 2nd January, 30th January, 20th March, 18th May and 21st May. PCR has contributed to diagnosis in all cases, but also virus isolation, clinical signs, liver pathology, IFAT, and gross signs. Furthermore 2 suspect cases.
On second case initially no clear signs of ISA disease, 4/30 PCR +ve, 6/30 virus +ve. Virus isolation has improved much since the outbreaks in the late 1990-ties.
The farms have to withdraw of fish within 3-6 weeks, and they are slaughtered under strict biosecurity.
Epizootic investigation: all live fish movements and other contacts over 12 months back from 2nd January 2009. There have been no movements of live fish for ongrowing outside the Southwest Shetland containment since mid June 2008, and none outside Shetland. All movements are investigated and spreading ruled out through inspection and testing.
Likely source and spread: The disease appears to have occurred after 27th June and spread hydrodynamically. The source is unknown, genetically the virus belongs to the European genogroup I, the HPR type is unique and novel. We have 2 theories: Either the disease is caused by a possible new emergence or by importation?
We have examined 216 wild freshwater fish and more than 1000 marine fish, all negative by qPCR.
We are aiming at regaining freedom from ISA. The industry have agreed on 6 months synchronous fallowing for the whole area, not just to eradicate ISA, but also to contribute to the control of sea lice.

Questions
***Brit Hjeltnes*:** Is anything previously known on the HPR0 situation in this area?
***Rob Raynard*:** No, not in this area. Mike Snow has tested for this app. 2 years ago. He identified 3 farms with HPR0, but that was in other areas.
***Brit Hjeltnes*:** Has the industry tested themselves?
***Rob Raynard*:** The industry has tested themselves in the autumn in relation to exports to Chile.
***Niels Jørgen Olesen*:** Did you type all the outbreaks, are they all the same HPR type?
Rob Raynard: We have tested 2 isolates until now and they are the same. The type is a completely new type.
Stig Mellergaard: What about vertical transmission?

***Rob Raynard*:** The evidence that vertical transmission of ISA occurs is generally very weak. In this particular case of ISA the eggs came from ISA free fish, and they are disinfected at arrival. The likely date of infection which is after 25 June 2008 does not support vertical transmission in this case.
Stig Mellergaard: Have you considered the human transportation?
***Rob Raynard*:** We have looked into wellboat movements.
***Brit Hjeltnes*:** It could be very interesting to have more information on the HPR0 type.
***Mike Snow*:** It is not a HPR0 type we have seen in Scotland before. Technically it is very difficult to type these isolates.
***Fiona Geoghegan*:** Have you had any resistance among the farmers, now the legislation has changed?
***Rob Raynard*:** Generally there has been good relationships but also some resistance, also because there is no reimbursement.

Tracing the spread of Infectious Salmon Anaemia virus (ISAV) in salmon farms in Norway

T.M. Lyngstad*[1], M.J. Hjortaas [1], P.A. Jansen[1], A.B. Kristoffersen[1], E. Karlsen[2], E.J. Johansen[3], C.M. Jonassen[1]
[1] National Veterinary Institute, Oslo, Norway, [2] Norwegian Food Safety Authority, Harstad, Norway, [3] Norwegian Food Safety Authority, Finnsnes, Norway

Abstract: Outbreaks of infectious salmon anaemia (ISA) were confirmed in 23 sea sites farming salmon in Norway in 2007-2008. Eleven of the outbreaks clustered in a local area in Northern Norway. In this study we present phylogenetic and sequence analyses of the Haemagglutinin-esterase (HE)-gene, including the hypervariable region (HPR), and the fusion protein (F)-gene of Norwegian ISAV isolates from 2007 to 2008. In addition, the study covers investigation for ISAV in 30 farms considered being at risk due to proximate location to outbreak farms (at-risk-farms). Organs from 10 fish from each outbreak farm and 30 fish from each at-risk-farm were screened for ISAV using Real Time RT PCR. The HE- and the F- genes of the virus were sequenced from positive samples. Phylogenetic analysis of the HE- gene, including the deletion pattern in the HPR, and the F-gene showed that 11 of the outbreaks that clustered in a local area in Northern Norway were closely related. ISAV was detected in 41 % of at-risk-farms, and the presumed low virulent HPR0 genotype of ISAV was more common than previously reported from Norway. Two of the eleven clustered outbreaks were sampled as at-risk-farms 3-4 months prior to ISA outbreaks. Phylogenetic comparisons between ISAV HPR0 and subsequent ISAV HPR from these two sites suggest that the different variants of ISAV represented independent infection events. We conclude that ISAV HPR0 is more abundant than previously shown in Norway. It is hypothesised that ISA outbreaks may arise from ISAV HPR0 mutating to virulent ISAV, and that virulent ISAV spreads horizontally resulting in small scale local ISA epidemics.

Minutes

In Norway ISA outbreaks have occurred widespread along the coast, and partly in small space-time clusters of sites. The number of sites included in this project is 23 ISAV outbreak sites and 29 at risk sites; at risk sites are sites in proximity to outbreak sites. Epidemiological data were gathered through standardised questionnaires. For screening, RT-PCR on segment 5 and segment 6 was used and ISAV was detected in 13 at risk sites.
Phylogeny on segment 6 and segment 5 showed nearly no variations in the examined fjord system. The isolates had identical deletions and were of the same HPR-type. One similar HPR-type was identified in an outbreak back in 2004.

HPR0 was isolated in 7 farms. On 3 sites HPR0 was isolated 3-4 months prior to ISA outbreaks.
We discovered it was easier to detect the ISAV on gill samples than in kidneys.
The low virulent sites have more variation than the outbreak sites when looking at segment 6. This is also true for segment 5, with even more variation.
A cluster of 11 outbreaks was identified with closely related HE genes including the depletion pattern in the HRP and F protein gene.
Low virulent HPR0 genotype is more common than previously reported from Norway.

Questions
Stig Mellergaard: Compared to avian influenza, we have a source of low pathogenic ISAV; can it be herring that transport the virus?
***Debes Christiansen*:** At the Faroe Islands we have tested a lot of herring, but have not found ISAV in any of them.
***Stig Mellergaard*:** When we test wild birds, we also seldom find positives.
***Niels Jørgen Olesen*:** What is done when you find HPR0 – is it then a case of suspicion?
***Trude Lyngstad*:** It is reported to competent authority but not treated as a case of suspicion.
***Debes Christiansen*:** Are there other epidemiologic clues, such as stress factors etc?
Trude Lyngstad: We should look more into this. Most outbreaks occur after 1 year at sea. We observed though one outbreak after only 2 months at sea.
***Neil Ruane*:** Have you noticed any effect of these measures on the spread of PD?
Brit Hjeltnes: It is hard to judge as we have no outbreaks of PD in northern Norway.

Fish health trends and developments in Norwegian aquaculture 2008

Hege Helberg* and Irene Ørpetveit
National Veterinary Institute

Abstract: In 2008, Norway produced 740 000 tonnes Atlantic salmon (*Salmo salar* L.), 80 000 tonnes rainbow trout (*Oncorhynchus mykiss*) and 14 000 tonnes Atlantic cod (*Gadus morhua* L.). The trend towards fewer and larger production sites for salmon continues. Viral haemorrhagic septicaemia (VHS) was diagnosed in two rainbow trout sites in 2007 and at two neighbouring sites in 2008. A marked increase in infection with *Flavobacterium psychrophilum* in rainbow trout has been detected. In farmed Atlantic salmon, an increase of outbreaks of ISA is the main finding. Pancreas disease (PD), heart and skeletal muscle inflammation (HSMI) and infectious pancreatic necrosis (IPN) continue to cause large losses, as do cardiomyopathy syndrome (CMS), proliferative gill disease (PGI) and winter ulcer disease. The detection of salmon lice (*Lepeophtheirus salmonis*) resistant to emamectin benzoate is a major concern.

Diseases in salmonids, number of sites diagnosed

	2003	2004	2005	2006	2007	2008
VHS	0	0	0	0	2	2
ISA	8	16	11	4	7	17
IPN	178	172	208	207	165	158
PD	22	43	45	58	98	108
HSMI		54	83	94	162	144
Piscirickettsia	5	0	0	1	1	1
Furunculosis	2	3	1	3	5	0
BKD	1	1	2	0	0	1
F. psychroph		3	1	2	2	16

The disease situation in cod is dominated by bacterial diseases, with francisellosis, vibriosis and atypical furunculosis being the major problems.

Diseases in Atlantic cod, number of sites diagnosed

	2005	2006	2007	2008
IPN	Not detect.	Not detect.	Not detect.	Not detect.
VNN (nodavirus)	Not detect.	3	6	3
Atypical furunculosis	3	13	9	16
Francisellosis	4	7	8	14
Vibriosis (*V. anguillarum*)	18	19	19	20
Cold water vibriosis (*V. salmonicida*)	2	Not detect.	1	1
Infection with *Vibrio ordalii*	1	Not detect.	3	Not detect.
Infection with *Vibrio logei/logei-like*	2	1	2	Not detect.
Infection with *Photobacterium* sp.	3	3	6	4

Minutes

Production: Atlantic salmon 740000 tonnes, rainbow trout 80000 tonnes, in all 1038 production sites. Cod 250 active sites, 13500 tonnes.
Trends: PD going up.
ISA: 17 outbreaks in 2008.
IPN: The disease is delisted, so we probably do not have the full overview. Most fish are vaccinated against IPN. Some outbreaks with large losses. App 45% of cases occurs in the hatchery phase, rainbow trout is almost exclusively affected during the hatchery phase.

PD is of huge concern; the farmers have begged the authority to list the disease, which it has now been since November 2007. In Norway we have SAV3. SAV1 (PD) as well as SAV2 (SD) have not been detected.
The disease has spread from Hordaland, but the middle part of the country is still free.
Heart and skeletal muscle inflammation (HSMI) in Atlantic salmon: infection trials indicates a viral aetiology. The disease is mainly seen in the first year after transfer to sea, but has in 2007 and 2008 also been diagnosed in hatcheries. Mortality 0-20%. Found all over.
VHSV genotype III diagnosed in farmed rainbow trout autumn 2007 and winter 2008. VHSV genotype III also detected in escaped rainbow trout within zone, but not in wild marine fish examined (n=260). 2007: 3 sites, 2008: 2 sites, all belonging to the same company.
There has been a dramatic increase of *F. psychrophilum* in rainbow trout in 2008. Systemic infections in 9 hatcheries with heavy mortalities and in 2 sea growing sites. There may be a common source.
Other disease problems: Cardiomyopathy syndrome (CMS), winter ulcer disease, proliferative gill disease.
Salmon lice resistance to emamectin benzoate confirmed on several sites. Multiresistance suspected on some sites. A new surveillance programme has started.
We have a new, emerging disease. It has been diagnosed in salmon in sea cages on south-western coast. Signs are variable mortality, loss of growth, no major known fish pathogenic agents detected. Macro: swollen and pale gills, yellowish liver, swollen spleen, circulatory failure, empty gut. Histology: gill and kidney lesions, hyperplasia of Bowman's capsule, haemorrhage, macrophage like cells in kidney, in heart epicarditis. Colleagues at Bergen University claim a Microsporidian is involved? Maybe associated with salmon lice as vector? Direct comparison is difficult as university of Bergen has done little histopathology.
Marine fish: mainly cod, 350 submissions from app. 85 sites. The main problem is *Francisella philomiragia* subsp. *noatunensis*. Reservoir for this pathogen can be found in wild fish. *Francisella* was listed in 2008. Íntracellular, chronic, granulomatous infection, all age groups are affected.
In 2009: PD 14 new outbreaks as of April, ISA 5 outbreaks, VHS no reoccurrence. An interactive map showing PD and ISA outbreaks is available at www.vetins.no as well as the annual report.

Questions
***Vlasta Jencic*:** Is piscirickettsiosis not an exotic disease, what have you done with it and how do you diagnose it?
***Hege Hellberg*:** We have had it for several years, but not of major concern and not exotic in Norway. It is diagnosed by histology and confirmed by PCR.
Niels Jørgen Olesen: *F. psychrophilum* what have you done about it? Others have had problem for years.
***Brit Hjeltnes*:** There seems to be a common source, a broodstock farm maybe. Information is put on our website. The Farmers have taken care of it by themselves. There have been proposals for listing of the disease.
***Hege Hellberg*:** The disease has mainly been a rainbow trout problem and there is a shortage of rainbow trout at the moment. Probably transferred from freshwater to the sea, where after horizontal spreading has taken place.

Monitoring of the viral fish diseases in Poland in 2004-2008

Jerzy Antychowicz[1],Marek Matras*[1], Ewa Borzym[1], Michał Reichert[2]
Department of Fish Diseases[1] and Department of Pathology[2] of National Veterinary Research Institute, Pulawy, Poland

Abstract: Fish Diseases Department of National Veterinary Institute exists for more than 70 years. For many years, we have carried out the monitoring of environmental, viral, fungal, and bacterial fish diseases. Since 2000, a regular diagnosis of the etiological agents of the notified fish diseases has been made. The viruses such as VHS, IHN, IPN, and SVC have been isolated at least in two of the following cell lines BF-2, EPC, RTG-2, FHM and identified by at least two of the following methods: ELISA, IFAT, PCR. KHV nuclear acids identification was made with PCR method using at least two of the following modification i.e. Gilad, Grey, and Bercovier. In some random cases, we isolated KHV in CCB cell lines and then we preceded onto virus electron microscopy (TEM) identification. Actually we are investigating on Real Time PCR application to KHV identification in various water temperatures.

The sampling was carried out under the principles presented in EU directives. The sampling was performed by district veterinary officers. The fish for the sample have been chosen according to the instructions prepared by prof. Antychowicz and authorised by Polish General Veterinary Officers. The sample consists of the live or sacrificed and cooled, below 10°C, fish, which were then delivered to our laboratory and immediately subjected to diagnostic investigations.

In 2000, prof. Antychowicz initiated the experiment and for the first time in Poland realized a program of controlling the VHS. It concerned 19 rainbow trout farms situated in one isolated river catchment. We made some achievements at the beginning with a substantial decrease in VHS cases though the region had the worst VHS on record in Poland. Unfortunately, we failed to eradicate the VHS completely, because some fish farmers participating in the programme did not comply with the established guidelines. Nonetheless, during the realisation of this programme we gained a lot of valuable experience, which could be utilised in the fulfilment of official programmes in the future.

Our practical experience gained in the experimental eradication programme and also during eight years of VHS and IHN monitoring, has shown that the main factor contributing to VHS and IHN introduction to Poland and spread throughout the country is unrestricted importation of live rainbow trout and its eyed eggs from other countries and inadequately control of live fish movement between Polish fish farms. It was caused because there were no regions in Poland officially free of VHS and IHN and no official programmes are actually realized. This situation appeared in spite of a great activity of veterinary service official veterinarian survey fish farms, select samples of the fish and eventually perform the eradication of VHS, IHN and KHV cases.

The second important factor involved in spread of VHS was ineffective disinfection of basins used by private proprietors for illegal fish transportation and improper neutralisation of the transport water before pouring it out.

It should also be stressed that the possibility of fish viruses spreading from one infected farm to other farms situated in the same river catchment increases when the distances between them decreases. One of our colleagues Dr. Mazur, a specialist of fish diseases, found that a distance of less than 1 – 2 km between salmonid farms, has a significant effect on the increased susceptibility the of the virus spreading through the water.

Two well-documented first cases of IHN in Poland were detected in 2008. In both cases, the infected fish originated from the fish eggs imported from western European countries, so it is

strongly suggested that the virus originated from the eggs – no cases of IHN were detected for many years in Poland before these eggs were imported.

Our practical experience gained in the experimental eradication programme initiated by Prof. Antychowicz (1999 – 2005) and also during eight years of VHS and IHN monitoring, has shown that the main factors contribiuting to spread of pathogenic salmonid viruses throughout Poland are the following:

- too short distances between salmonid fish farms – that appears as a result of dynamic fish farming development in our country,
- ineffective disinfection of transport tanks used for fish transportation and improper neutralization of the transport water before pouring it out after fish transportation,
- unrestricted importation of live rainbow trout and its eyed eggs from other EU countries and inadequate control of live fish movement between Polish fish farms caused by lack of any EU regulations in the case of the countries or regions that are not officially free of the VHS and IHN.

Concerning KHV, the following factors facilitate the introduction and spread of this disease in Poland:
- uncontrolled movement of live koi carp between the countries throughout EU territory,
- stocking koi carps with carps reared for consumption together in production ponds
- propagation of crossbred between ordinary carp and koi carp for consumption purposes and keeping them together with ordinary carp,
-lack of realistic long-term programmes for the KHV control in the traditional large carp farm environment with complicated systems of water facilities feeding the ponds which make the eradication of the disease in some region in Poland very difficult,
-lack of any EU Directive regulating the movement of live carp and koi carp between the farms that did not have status free of KHV.

As it was stated there were no official programmes realized in Poland for controlling VHS, IHN and KHV for the purpose to obtain official free status in river catchments or in farms. The only way so far to control fish viral diseases applied in Poland is regular monitoring and disease eradication in each infected fish farms, but the other farms in the river catchment are usually not considered in eradication. We found these methods not effective enough as we can see from the presented figures. The regular monitoring of VHS, IHN and KHV could be anyway a good beginning for the implementation of official programmes in the future. The following conclusions could be made:
- the realization of the official programmes is connected first of all with good management fishery practices among others in the proper disinfection of transport vehicle basins used for fish transportation and purchasing by the farmers only certified fish from officially free farms and regions
- the realization of the long-term VHS, IHN and KHV programmes is urgent in order to decrease the spread of these diseases in Poland
- viruses genotyping together with the geographic information system (G.I.S) should be applied in the near future for efficient monitoring of the important fish viral diseases and for tracking the sources of infections especially when there is suspicion that in particular case virus could be introduced from abroad.

I hope that our remarks on the factors, which contribute to the spread of fish viral diseases will be helpful for countries, which like Poland, are going to start with the official programmes of VHS, IHN, and KHV eradication on their territory.

Minutes
There are 442 salmonid fish farms and 1293 carp farms in Poland giving rise to an approximate production of 18.000 tonnes of rainbow trout and 18.000 tonnes of carp. Since 2000, a survey for listed viral fish diseases have occurred in Poland. Sampling has been processed according to EU standards. Most outbreaks of VHS have occurred in the North West of Poland. One factor contributing to VHSV and IHNV introduction to Poland is unrestricted importation of live rainbow trout and eggs. Another is ineffective disinfection of transportation tanks. Furthermore, when distances between farms are less than 2 km an increased risk of VHSV spreading is observed.
Concerning KHV, most cases have been observed in the south of Poland where most carp farms are located. Factors that can contribute to the introduction and spread of the disease are e.g.: uncontrolled import, stocking of koi carp together with consumption carp.

Questions
Giuseppe Bovo: You had two outbreaks of IHN in 2008. Can you import eggs from any kind of farm?
***Marek Matras*:** No, imports only occur from certified disease free farms.
Olga Haenen: Concerning the IHN outbreak in Holland, eggs were bought with certificate from a certified farm. However, the transportation company probably had problems with ineffective disinfection of transportation tanks.
Marek Matras: Import was from certified farms from Western European countries.
Fiona Geoghegan: Does the industry consider legislation as a positive thing?
Marek Matras: Yes, in general they do. It is now easier to get samples and to carry out legislation.

Eradication of IPN in a farm with chronically infected stocks

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Abstract: In rainbow trout from a commercial fish farm producing fish for human consumption IPNV was regularly detected over a period of several years. This resulted in restrictions for the farm as IPN is a notifiable disease in Switzerland. Although the farm received water from a river, it was suspected that the source of virus was the introduction of infected fish imported into the country rather than river water. These imported fish were regularly found to be positive for IPNV. To eradicate the infection from the farm a stepwise procedure was initiated which allowed the farmer to continue production without interruption. To this end series of tanks were disinfected and fallowed before stocking with new fish from a source certified as IPNV-free. This procedure was continued until all fish of the original stock had been replaced. As additional measures newly introduced fish were regularly examined for the presence of IPNV and random samples from all tanks stocked with new fish were tested. All tanks but one stocked with new fish showed to be negative for the whole production period. In the one tank, where the virus had been detected in newly introduced fish no fallowing had been performed after disinfection of the tank. Since 10 months no infected fish could be detected any more. This result clearly indicates that the source of virus was not the river water and that eradication of IPN in a commercial farm is possible, given some conditions are met.

Minutes
IPN is a notifiable disease in Switzerland. In one particular farm - producing market sized rainbow trout (100 t/year) - IPNV positive fish were repeatedly identified. In this farm fish were located in

round tanks/raceways. Two possible sources of infection: from the water system or most probably from imported fish. The consequence was, in agreement with the farmer, that sale of live fish was prohibited and sanitation demanded. A change of fingerling supplier was made and a stepwise sanitation of the farm was set-up. The duration of the sanitation was one year after which all samples were negative, a good growth of new stocks and low mortality rates were observed. All restrictions were lifted and increased production numbers were observed. The absence of new infections at the farm strongly indicated that the cause of infections have been because of import of infected fish.

Questions
Birgit Oidtmann: Does the number of cases reflect those isolated from the same farm?
Thomas Wahli: No, normally we see one or two farms infected
***Olga Haenen*:** IPN can be difficult to get completely rid of. Do you think it could break out again?
***Thomas Wahli*:** The virus may still be present at very low levels. We have not been able to detect it in our laboratory. But we can not be 100 % sure that it is completely gone.
***Sven Bergmann*:** Have you checked if the fish contains antibodies?
***Thomas Wahli*:** No
***Guiseppe Bovo*:** IPN can be vertically transmitted. Can this farm sell fish for restocking?
***Thomas Wahli*:** It is not allowed. This farm can only sell for consumption.
***Niels Jørgen Olesen*:** How long time will it take before this farm can be claimed free of IPNV?
***Thomas Wahli*:** We have not seen IPNV since last year. New fish are growing well. Regular testing for viruses will occur.
***Niels Jørgen Olesen*:** What kind of disinfection have you performed at the farm?
***Thomas Wahli*:** Several, formalin, Virkon S and heating the tanks
Irene Ørpetveit: A comment on sensitivity of IPNV detecting methods: In our lab, we find the sensitivity of real-time PCR and cell lines almost similar.

Total eradication of VHS in Denmark

Henrik Korsholm
Danish Veterinary and Food Administration.

Abstract: Since the first appearance of VHS in the 1950-ties the disease was rapidly spread to all of Denmark by trade of fish. Targeted eradication has been carried out under official supervision and control since 1970. This has resulted in a decrease in the number of infected farm, but not to a total eradication of the disease. A project for final eradication of VHS has been initiated in March 2009 and is planned to run for 5 years. Participation in the project is mandatory for the fish farms. A grant from the European Fishery Fund finances the project, which has a total budget of 6.5 million €.

Minutes
The first observation of VHS in Denmark was in the 1950-ties. In the 1960-ties a private voluntary eradication program was started. In 1970-ties an official control program for VHS eradication was started and in 2009 the final eradication of VHS in Denmark will hopefully occur. For fish farms considered to have a potential risk of being infected by VHSV, the method applied is stamping out (removal of all fish and gametes, cleaning and disinfection, fallowing, restocking with healthy fish). The Skjern Å systems and Ringkjøbing fjord is a special high risk area. According to the new directive, fish farms have to be categorised. Danish fish farms free of VHSV will be put into category I whereas the rest are put into category II. Application for an eradication-programme was

submitted in May 2008 and approved by the European Fisheries Fund in autumn 2008. The total budget is 48.5 mill DKK = 6.5 mill €. In the program: It is mandatory to stamp out; taxation and compensation are provided to the fish farmers according to the value of the fish; cleaning, disinfection and production stop are carried out by the fish farmers.

Questions

Birgit Oidtmann: How did you calculate the amount of food for which the compensation of 0.33 €/kg feed for fallowing was given?

Henrik Korsholm: Each fish farm has been appointed a maximum feed amount allowed to use by the Danish Authorities. When an outbreak occurs, compensation is calculated on behalf on the value of the fish, the day before the outbreak occurred.

Drazen Oraic: How do you remove rainbow trout from a river system?

Henrik Korsholm: By electrofishing. The rainbow trout are removed from the river system whereas other fish are left. Sometime repeated electrofishing occur.

Giuseppe Bovo: Are rainbow trout in the sea coming back to the river systems and do rainbow trout breed in the river systems – if yes it could cause problems.

***Henrik Korsholm*:** Yes, escaping rainbow trout from sea farms are going to freshwater systems and there are indications that the rainbow trout has started breeding in Danish river systems.

***Giuseppe Bovo*:** What is the prevalence of infected feral fish?

***Henrik Korsholm*:** It is low but it has not been determined exactly.

Fiona Geoghegan: The project is financed by a grant from the European Fishery Fund that ends in 2013. Is that why the project runs out in 2013?

***Henrik Korsholm*:** Yes.

Fiona Geoghegan: And the Danish ministry has to supplement by paying 50%?

***Henrik Korsholm*:** Yes.

***Niels Jørgen Olesen*:** Maybe this is the first of such kind of a programme to receive funding from the European Fishery Fond.

***Stig Mellergaard*:** A similar programme from Germany has received support.

Isolation of an Iridovirus (Ranavirus) from catfish *Ameiurus nebulosus* in Hungary

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Abstract: Iridovirus infection was identified in catfish (brown bullhead), *Ameiurus nebulosus* (*Ictalurus nebulosus*), showing severe clinical signs. In May 2008 intense bullhead mortality was observed in a four-hectared lake which belongs to a fishing club. No clinical signs were seen in other fish species living in the same lake, such as carp, pike, pikeperch, and grasscarp. The diseased fish showed distinct clinical and histopatological signs of Iridovirus infection: petechiae on the surface of the body and petechiae and necrosis in the internal organs. A cytopathic virus was isolated from homogenised spleen, liver and kidney on both EPC and BF-2 cells. Inclusion bodies were seen in the cytoplasm of the infected cells using a haemathoxilin-eosin staining method.

The virus was identified by PCR methods. A 580 base pair length PCR fragment spanning the MCP gene was amplified from the organ suspension and tissue culture supernatant by using primers and protocol described by A.D. Hyatt et al. in 2000. Homology searches of GenBank revealed 100% amino acid identity with corresponding MPC sequences of the catfish and sheatfish iridovirus.

Minutes
The presentation was on the first report on the occurence of a disease caused by European Catfish Virus (ECV) in Hungary (sequencing of a PCR fragment of the MCP gene showed 100% identity with ESV and ECV). The virus could induce considerable mortality in Brown bullhead (*Ameiurus nebulosus*) but no disease was seen in other species living in the same lake. Water refill had been performed from backwater 1 week before the outbreak. The source of the infection was not determined. Possible explanations could be that virus carriers could have come into the reservoire from the backwater during the water refilling or might originally be present in the lake, and the changing of water quality have generated the epidemic or maybe both.

Questions
***Sven Bergmann*:** At what temperature started the fish dying?
***Tamas Juhasz*:** At 20–22°C but it was not exactly determined.
***Olga Haenen*:** The rana iridoviruses are warm water viruses with optimum growth at 24°C
Tamas Juhasz: Yes, but we also see that they can grow at 18°C and can induce cytopathic effects at this temperature.
***Giuseppe Bovo*:** Do you have the similar species *Ictalurus melas*?
***Tamas Juhasz*:** No we only have *Ictalurus nebulosus*

Koi Herpesvirus Disease: Data from the global EPIZONE questionnaire 2009

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Abstract: EPIZONE is a big EU network of excellence project within FP6 (www.epizone-eu.net), with 20 partners from Europe, and China, Turkey, FAO and DiVa. Its mission is to improve research on preparedness, prevention, detection, and control of epizootic diseases within Europe to reduce the economic and social impact of future outbreaks of emerging/notifiable diseases, like Foot-and-mouth disease through increased excellence by collaboration.

Within EPIZONE, Work package 6.1 covers emerging diseases of fish, including Koi Herpes virus (KHV), which causes the notifiable KHV disease (KHVD) in koi and carp *(Cyprinus carpio).*

In Sept 2007, at the last EAFP Conference in Grado, results of the detailed EPIZONE questionnaire on KHV disease in 2006-2007 were presented.

In March 2009 a follow up KHV questionnaire was sent to > 65 countries world wide. By the start of May 2009, 40 countries had responded, i.e. > 60%. The results of the KHV questionnaire will be presented, including the trends compared to the 2007 questionnaire.

Questions of the questionnaire were about koi (1), cultured (2) and wild carp (3), all *Cyprinus carpio*:

- Prevalence of KHV in your country? Year of first detection? Number of outbreaks in 2004-2009 in 1, 2 or 3?
- Clinics: what clinical signs were present in KHV outbreaks?
- Outbreaks: Was there disease and mortality in small and/or big fish?

- Diagnosis: Which diagnostic tests were used for KHV detection, screening and confirmation? Did/do you participate in the KHV PCR ring test of CEFAS (UK);
- Susceptible fish species: Was KHV isolated from other species than koi/carp?
- Latent carriers: Do you have any experience with latency of KHV in koi/carp?
- Measures (stamping out, temp change, therapy) and effects in 1, 2, and 3?
- Vaccination: Is a KHV vaccine used in your country?
- Any research on KHV in your country and laboratory?
- National legislation in your country?
- Any Further points?

A full updated literature list on KHV on request: please E-mail olga.haenen@wur.nl

Minutes

The first Epizone KHV disease questionnaire was sent to 67 countries in December 2006 - January 2007; the second was sent to 72 countries in 2009. Many European countries have the disease, some have not and there is also some question marks. On a world wide scale the virus is observed in several continents – Asia, North America and Europe whereas it has not been detected in Australia. Since the first questionnaire, the disease have been identifies in several new countries, e.g. Canada, India, New Zealand, Slovenia and Guatemala. In 2007: 17 countries found KHV in koi carp, 12 in cultured carp and in 4 in wild carp. In 2009: 22 countries found KHV in koi, 10 in cultured carp, and in 5 in wild carp. In 2007: The mortalities varied: in koi 10-100%, cultured carp <10%-100%, and in wild carp mortalities were often high but unknown. In 2009: similar numbers, and 5-95% mortality in wild carp. In 2007: 13 countries had no KHV tests yet; in 2009: 12 have no tests. In such cases, samples may be sent to other countries for KHV testing. KHV may be detected also in species such as crucian & prusian & grass carp, goldfish, ide, *Anacistrus* sp., bream, sturgeon, sheatfish, etc. Possible latency reported from 10 countries. Measures at KHV outbreak: Stamping out, disinfection, stop fish movements, water temperature raised to 28-30°C, vaccination etc. In 2009: 28 countries had legislation on KHV, so many maybe because of 2006/88/EC.

Questions

***Neil Ruane*:** Those countries that are negative for KHV, do they have an active surveillance programme or no surveillance programme?
***Olga Haenen*:** In most cases there is no surveillance programme.
***Sven Bergmann*:** A comment on the vaccine from Japan, Prof. Miyazaki's group, it should work very well.
***Giuseppe Bovo*:** What about the formalin killed vaccine – is that used?
***Olga Haenen*:** It is not officially allowed to use live vaccines in Europe. The reason is that one is afraid they can mutate back to the aggressive type.
***Fiona Geoghegan*:** There is no licence in Europe to use these vaccines.
***Stig Mellergaard*:** An approval to use a vaccine in the EU can be provided in case of an emergency programme.
***Keith Way*:** The EU has to tell which diagnostic test is approved, e.g. which PCR test – is there any news on that?
***Olga Haenen*:** We should ask Sigrid tomorrow.
Birgit Oidtmann: In Scandinavia there is not a big carp industry – what is your opinion?
***Brit Hjeltnes*:** We do care – but it is not a big concern for the industry at the moment but it might be in the future.
***Birgit Oidtmann*:** What about countries where categories are not so important - Can they stay in category 3 or 4?

***Stig Mellergaard*:** It is not meant that countries should stay in these categories - countries can use the opportunity to apply for freedom for historical reasons if it is relevant.
***Olga Haenen*:** If koi import has occurred from Israel – we can not say if fish has KHV, as we cannot distinguish the vaccine strain from the field strain.
***Fiona Geoghegan*:** Is it possible to differentiate your results into cases of KHV in open systems versus cases in closed systems, since this is the way the legislation is now formulated?
***Olga Haenen*:** I will see if that can be done, as it would make the data more meaningful, under the circumstances.

Epidemiology and combat against fish epidemics - thoughts on koi herpesvirus disease (KHVD)

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Abstract: Since December 2005 koi herpesvirus (KHV) disease (KHVD) has been notifiable for carp (*Cyprinus carpio*) and, as a consequence of the severe economic losses especially in cyprinid aquaculture, since 2006 also for koi (*C. carpio*). Carp farmers in different federal states of Germany have no possibility to interfere as a new virus is reducing their animals.
The lack of understanding of the epidemiological correlations of this herpesvirus-induced disease, the limited diagnostic possibilities as well as the extensive form of carp aquaculture in Germany often lead to misinterpretations of the epidemic situation after negative test results.
Every year more farms, pet shops, traders but also wild and non-cyprinid fish are affected by KHV. Nevertheless, clinical symptoms are only found in *C. carpio*.
KHVD seems to be a very seasonal disease. Most cases of KHV and KHVD are detected between June and September. For diagnostics, only molecular tools are suitable because other assays, e.g. virus isolation in cell culture followed by immunofluorescence, are not sensitive enough for virus detection.
To develop an adequate strategy to combat the epidemic it is crucial to assess the actually occurring KHV prevalence in infected or latently infected compartments. At present, there is no legal obligation to combat the disease in Germany. The regional authorities decide individually about the measures to be taken. The options range from stamping out after an acute outbreak with zero prevalence in a farm or pond to immunization strategies which also include escaped animals. The latter measure is most effective when the KHV prevalence is high.
The number of sampled fish and the sampling method (an active targeted sampling is recommended) can strongly influence the results of the diagnostic tests as well as the consequences. False-negative health certificates often lead to the statement "free of KHV" when no positive sample is found by e.g. PCR or *realtime* PCR. In contrast, when only one sample out of 100 is safely considered to be KHV positive, doubts remain.
The general characteristics of herpesviral infections, i.e. the fact that they induce persistence or latency, often are not taken into account. In addition, the situation in possible carrier fish and the education of the hobbyists are neglected.

Minutes
KHV disease is a notifiable disease for carp and it can cause severe loss for the cyprinid aquaculture. It is important to understand the epidemiological relations of the disease. KHV is a seasonal disease, primarily detected between June and September. As diagnostic tools, the

molecular methods are most suitable as they are the most sensitive. It is crucial to assess the actual occurrence of the virus in infected or latent infected compartments.
The level of virus in a fish is unpredictable. Therefore, it is desired that the virus gets a chance to grow up in the fish before sampling. 24 hours in stressing conditions would make the virus grow in the fish. If no stress is applied to the fish, the diagnostic methods might not be sensitive enough to detect the virus. You will never find 100% infected animals in a population. That is a big problem for random sampling approach. Never mix fish with ornamental fish.

Questions
***Stig Mellergaard*:** Concerning latent carriers, it is impossible with eradication unless using uninfected stocks.
***Sven Bergmann*:** Vaccination does not eliminate infection from wild type KHV. Furthermore, the wild type grows faster than the vaccine virus.

SESSION II: Technical issues related to sampling and diagnosis

The 2008 KHV PCR methods ring trial

Keith Way
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Abstract: Following successful KHV PCR methods ring trials in 2006 and 2007 the 2008 trial further expanded to include 44 laboratories in 32 countries. The format was similar to the 2007 trial where participating laboratories were requested to use their preferred DNA extraction method and to trial the two PCR protocols recommended in the OIE diagnostic manual chapter on KHV disease. They were also expected to use a real-time PCR assay or a nested-PCR assay to analyse the samples. The main difference for 2008 was that one of the samples was spiked with a cyprinid herpesvirus (CyHV) that was not KHV and laboratories were then asked to test the samples using a protocol that included generic primers that target the CyHV polymerase gene.
All of the sample vials contained lower amounts of virus DNA and total DNA than in previous samples making the 2008 ring trial more technically demanding than the previous trials. As a result, a much larger proportion of labs reported incorrect results (false positives and negatives) than in previous ring-trials. False positive results were reported by 18 laboratories, with one or more of the assays used. This suggests that they may have cross-contamination and sample handling issues in the labs they use for their molecular virology analyses.

A more detailed analysis of the 2008 ring trial results will be described and the format of future international ring trials or proficiency tests will be discussed.

Minutes
31 of 43 labs achieved clean/correct results with at least 1 PCR assay. 25 of 43 labs reported incorrect results. 19 labs reported false positives. Real-time PCR appeared most reliable. No more funding for doing the ring test is available. Collaboration with VLA in UK on future ring tests is a possibility. The cost of this ring test is to be negotiated.

Questions
***Rob Raynard* and *Søren Kahns*:** Is it generally the more experienced PCR labs that do real-time PCR - could this explain why real-time PCR appeared more reliable?

***Keith Way*:** It is generally the most experienced labs that use real-time PCR but a connection between this fact and the observed reliability of real-time-PCR has not been investigated.
***Debes Christiansen*:** What about results from negative controls do they also show a lot of false positives?
***Keith Way*:** Results from the labs in-house negative controls are not known.

Application of controls that minimises the risk of obtaining false-positive or false-negative results in diagnostic PCR assays

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Abstract: In order to assure a high reliability of the PCR used for routine diagnosis, special precautions should be applied in order to prevent false-positive or false-negative results. False-positive results may arise from cross-contamination from positive samples but most often arise from carry over of PCR products from earlier experiments. False negative results most often arise because of technical (e.g. pipetting) errors or presence of inhibitory factors.

Using the PCR that is based on the Bercovier TK primers for detection of Koi Herpes Virus (KHV) as an example, we will illustrate how controls could be used in a diagnostic PCR setup in order to minimise the risk of obtaining false-positive or false-negative results.

Today, artificial genes are commercial available for reasonable prices. We designed a DNA template (KHV_Pos_2) containing the TK primer sites but flanking a DNA fragment of different size than the region amplified from the KHV genome. In order to reduce the risk of obtaining false positive results, we use this KHV_Pos_2 template as the positive PCR control and the positive purification control because carry over contaminations can be discriminated form a true positive from the sizes of the amplified DNA fragments.

In order to validate the correctness of our negative results, we use a setup, where our samples are spiked with the KHV_Pos_2 template prior to DNA extraction. This allows co-extraction of templates. The identical TK primer sequence in the KHV_Pos_2 template allows co-amplification in the same tube and the size differences allows discrimination. This permits monitoring of the performance of quality of the DNA extraction procedure. However, the presence of two templates in one reaction tube can cause competition that might reduce analytical sensitivity. In order to be sure that a positive signal is not lost because of competition, we analyse our samples in duplicates of which only one of the duplicates is spiked with the KHV_Pos_2 template. Furthermore, in order to minimise reduction in sensitivity, the one duplicate is spiked with the KHV_Pos_2 template in a concentration slightly higher than the detection level.

Questions
***Sven Bergmann*:** Is it wise to use competition with control plasmid when several samples are near detection limit already?
***Søren Kahns*:** We use "duplicates" where only one sample is subjected to competition.
***Birgit Oidtmann*:** It might be that the plasmid serving as extraction control could be easier extracted than KHV DNA.
Søren Kahns: Yes, the control is not bulletproof but in my opinion much better than not using anything.
***Heike Schütze*:** Why not use an internal gene as control?

***Søren Kahns*:** No obvious candidate has been available. It will probably be hard to find a candidate expressed stably and in low enough amounts to avoid substantial competition.

Implementation of a one-tube assay for koi herpesvirus (KHV) detection adapted to latent infected carrier fish

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Abstract: Koi herpesvirus (KHV, syn. CyHV-3) is an emerging disease agent in cyprinid aquaculture world-wide. In Germany, outbreaks of KHV disease (KHVD) and detections of the causative viral agent increased in both edible carps and imported ornamental kois from 2002 to 2008. In extensive carp aquaculture, mortality rates of up to 80 - 100% occurred in 2008 in harvested fish but also in koi (both *Cyprinus carpio*). As a consequence, various fish farms suffered severe financial losses (200.00 and 900.000 €), which in some cases led to the financial collapse of these farms.
One special problem is the behaviour of KHV which obviously induces persistence in infected, but healthy appearing fish. In this phase of viral manifestation, a very weak virus load (5 – 10 particles) can be found in kidney and leukocytes, sometimes also in gill tissue. Most of the currently available diagnostic tools are not sensitive enough to detect these weak virus concentrations. These unrecognized infected animals represent a real threat for naïve, non-infected carps or kois of any size or age.
To overcome this problem, a new molecular assay has been tested and its diagnostic sensitivity and specificity have been compared with other commonly used diagnostic methods. As "golden standard" served a quantitative *duplex realtime* PCR (modified according to Gilad et al. 2004 and Hoffmann et al. 2006) including internal and external controls. This assay permitted an absolute determination of the KHV DNA content in the different sample tissues and controls.
The diagnostic sensitivity/specificity of conventional PCRs, nested PCRs, one commercial LAMP and *realtime* PCRs were directly compared showing the content of "copies/reaction".
In individual samples of latent KHV infected animals we also found that the KHV DNA content often ranged between 5 and 10 genomic equivalents or copies/reaction. To minimize the contamination risk in the laboratory caused by the routine use of nested PCR and to reduce the expenditure of time and effort, a one-tube semi-nested PCR (sn PCR) was established. The test reached an equal diagnostic sensitivity of 5 to 10 copies and is specific for KHV DNA only. As heterologous virus or DNA controls carp pox virus (CyHV-1) DNA, goldfish haematopoietic necrosis virus (CyHV-2) DNA, Channel catfish herpesvirus (CCV, IcHV-1) DNA and *herpesvirus anguillae* (HVA, Ang-HV 1) DNA were utilized. No signals occurred in gels after sn PCR or in *duplex realtime* PCR results with these DNAs.

Keywords: KHV, persistence, DNA content quantification, semi-nested PCR

Main literature:

Gilad O, Yun S, Zagmutt-Vergara FJ, Leutenegger CM, Bercovier H, Hedrick RP (2004) Concentrations of a koi herpesvirus (KHV) in tissues of experimentally infected Cyprinus carpio koi as assessed by real-time TaqMan PCR. Dis Aquat Org 60:179–187

B. Hoffmann, K. Depner, H. Schirrmeier and M. Beer (2006) A universal heterologous internal control system for duplex real-time RT-PCR assays used in a detection system for pestiviruses, *J Virol Methods* **136**: 200–209.

Minutes
Only one PCR alone seems not to be sufficient. There has been an agglomeration of a Bercovier's TK PCR negative KHV strain in Germany. Latent infected fish are often not detected (a stress model is necessary). KHV- free certificates are often false-negative. Pooling samples are "deadly" for diagnosis of latent/persistent infected fish. Sensitivity and specificity of PCR results after lethal and non-lethal sampling are comparable. Best PCRs: Gilad's realtime, nested PCRs and semi-nested PCR.

Questions
***Søren Peter Jonstrup*:** Did you test the effect of extraction kits?
***Sven Bergmann*:** Yes. Qiagen kit works very well.
***Birgit Oidtmann*:** It would be nice to share information between labs about technical issues.
***Brit Hjeltnes*:** Could such information be collected on the CRL-website?
***Guiseppe Bovo*:** Could you comment on your experience with pooling samples?
Sven Bergmann: Pool no more than 2 fish to discover latent infection.
***Søren Kahns*:** Are the TK primers all right to use?
***Sven Bergmann*:** There could be a population of KHV not recognized by TK-primers.
***Keith Way*:** We recommend the TK primers, but Sven Bergmann may have got information that changes the situation.

Development of a robust accredited real-time PCR laboratory system and its application to the routine detection of fish pathogens

Mike Snow*, Nicola Bain, Fiona Doig, Julia Black, Rebecca McIntosh & Holly McKay
Marine Scotland Marine Laboratory, 375 Victoria Rd, Aberdeen, AB11 9DB Scotland.

Abstract: In recent years, Taqman real-time PCR (qPCR) has increasingly become the molecular method of choice for diagnostic laboratories involved in the routine detection of pathogens of aquaculture. The methodology builds on the principles of conventional PCR but offers significant additional advantages which are of relevance in the diagnostics context including:
i) The specificity conferred by the requirement for binding of both primers and highly specific probe in a single assay.
ii) The sensitivity conferred by the fluorescent detection chemistries employed.
iii) The speed of processing due to the lack of requirement to visualise PCR products.
iv) The potential to gain quantitative data, aiding interpretation of results.
v) The potential to automate and thus include a variety of procedural controls.
Despite the potential advantages of qPCR-based diagnostics, practical implementation and standardisation of the technique in fish health laboratories has not been without its problems. Such problems have been largely focussed around issues such as how to compare, standardise and interpret results (Ct numbers) both within and between laboratories, especially when apparently very low levels of pathogen genetic material are detected. This problem is exacerbated as qPCR tests can often report positive results which may not be supported by alternative tests conducted in parallel.
As with development of any diagnostic test, key to maximising the potential benefits, maintaining credibility and avoiding pitfalls in routine applications is the development of a robust and reliable system. We report here our experience in development of a system for the development and application of qPCR based diagnostics at Marine Scotland. Here at the National Reference Laboratory for fish, shellfish and crustacean diseases in Scotland, we were among the first to develop independent accreditation of qPCR testing based on accreditation of a generic qPCR methodology, encompassing assay design, validation and operational guidelines.

Minutes
qPCR is a potentially sensitive, specific and high throughput diagnostic method. Routine application of qPCR in the diagnostic laboratory requires careful consideration to the prevention of contamination, experienced and trained personnel, inclusion of extensive and appropriate controls to ensure correct interpretation of results, validation and preferably accreditation, and careful interpretation and verification of result where possible/appropriate (e.g. by sequencing). Mike Snow iss happy to share information on how they validated qPCRs.

Questions
***Olga Haenen*:** Do you start by diluting suspected highly concentrated samples?
***Mike Snow*:** No but they are treated differently in the lab to avoid them contaminating other samples.

General discussion on PCR
***Søren Kahns*:** Should we declare conventional PCR dead and only go for real-time PCR?
***Mike Snow*:** Real-time PCR is not the universal solution. There are still cases where a conventional PCR is the best or only available choice.
***Keith Way*:** There are research samples, diagnostic samples, and surveillance samples. Should we go for 3 streams of sampling handling in the lab?
***Mike Snow*:** That would be optimal, but hard to achieve. We have enough work just having two streams.
***Søren Kahns*:** We have seen that factors like qPCR kit, extraction kit etc. can influence the result.
***Sven Bergmann*:** We have also experienced this on KHV PCR.
***Keith Way*:** OIE does not like recommendations of kits even though this may be a quite important factor.
***Sven Bergmann*:** We recommends β-actin as internal standard in qPCR. We have successfully tested this in 25 species.
***Niels Jørgen Olesen*:** We have to decide on good diagnostic method to diagnose KHV for inclusion in guidelines in a new Commission Decision on sampling and diagnostic procedures for KHV. A workshop will be held in NL in November 2009 in EPIZONE regime in order to propose final recommendations.
***Keith Way*:** My favourite is Gilad qPCR. As conventional PCR I recommend nested TK, but Sven Bergmanns results might interfere with this.

Serology Part

Results from an inter-laboratory proficiency test on detection of antibodies against VHSV and IHNV in rainbow trout

J. Castric*[1], C. Quentel[1], J. Cabon[1], F. Lamour[1] and N. J. Olesen[2]
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[2]Community Reference Laboratory for Fish Diseases, Technical University of Denmark, Aarhus, Denmark

Abstract: The main objective of the inter-laboratory proficiency test organized in 2008 by Afssa and DTU Vet in the frame of EPIZONE was the development of robust serological techniques for detection of specific antibodies against VHSV and IHNV. Eight participants from different European laboratories agreed to participate to the test and were asked to check the presence of

antibodies against the two viruses by seroneutralisation techniques (SNT) or by ELISA in thirty rainbow trout sera. Two participants agreed to compare two different SNT, a 50% plaque neutralization test and an end-point serum neutralisation test and five participants applied both SNT and ELISA on all or part of the sera. The sera used in the test were obtained from trout experimentally infected with VHSV and/or IHNV or originated from an infected farm. All participants used the same viral strains, complement, as well as positive and negative control sera.
The results obtained using the two serological methods were in good agreement with those expected, especially in the laboratories where the techniques have been implemented for many years. Differences arose mainly between results concerning sera having low titres but with some exceptions, the same sera were found positive using the two techniques. When comparing the two techniques used in three laboratories for detection of IHNV antibodies, SNT appears more sensitive and more specific that ELISA. With VHSV, SNT was more specific as no false positive was detected by three participants while some negative sera were found positive by ELISA. Additional results obtained using the two techniques applied to the same samples will be necessary before concluding on the sensitivity and specificity of the two techniques.
Both ELISA and SNT can be used to detect specific antibodies against VHSV and IHNV in trout sera at the level of a fish population. The choice of the technique depends on each laboratory practices.

Minutes
Main objective of this inter-laboratory proficiency test is to develop and validate serological techniques (ELISA and SNT) for detection of specific antibodies against VHSV and IHNV. Participants were asked to check for the presence of antibodies against VHS and IHN viruses in the 30 sera. Furthermore, 2 participants agreed to compare 2 seroneutralisation techniques: 50% plaque neutralisation and end-point neutralisation. 5 participants performed ELISA on all or part of the sera. 4 participants performed SNT and ELISA on total or part of the sera. In conclusion both ELISA and SNT can be safely used to detect specific antibodies against VHS and IHN viruses

Questions
***Birgit Oidtmann*:** Regarding the IHNV antibodies – did you have specificity problems?
***Jeanette Castric*:** Only a few labs found false positive samples. In general less concordance between the results with sera having low neutralising titres.
***Brit Hjeltnes*:** For how long time will a population of rainbow trout stay positive for antibodies in the sera?
***Jeanette Castric*:** We have found antibodies at least one year after infection.
***Niels Jørgen Olesen*:** Positive sera can be found in a population at least one year after an outbreak. Infection depends on the temperature. If fish gets infected at low temperature there might be delayed onset, up to three months. This can cause a problem for surveillance.
***Sven Bergmann*:** We sometimes observe problems with our ELISA plates – Nunc Polysorb where some wells work OK whereas some does not. Are there any other having such problems?
***Jeanette Castric*:** We use same NUNC ELISA plates and do not see such problems.
***Sven Bergmann*:** We observe the same problem with other viruses.
***Niels Jørgen Olesen*:** We have tested different ELISA plates, in our hands Macrosorb (Nunc) are the best.

Optimisation and validation of an ELISA for detection of antibody to cyprinid herpesvirus 3 (CyHV-3, KHV)

Keith Way
Cefas Laboratory, The Nothe, Weymouth, Dorset, DT4 8UB, UK.

Abstract: An ELISA was developed for detection of serum anti-KHV antibodies in populations of experimentally exposed common carp (St Hilaire et al. 2009). However, a thorough validation was required before the ELISA could be used to assess previous exposure to KHV in natural populations of carp. During the early stages of the validation, minor improvements were made to the KHV antibody ELISA and further optimisation of the method produced a more robust test that could be performed by less experienced technicians. The test was shown to have good repeatability between technicians in the same laboratory and in different laboratories.
Cross-reaction with Cyprinid herpesvirus-1 (carp pox) antigens was observed at serum dilutions of 1/200 and 1/400. To achieve the necessary analytic specificity higher serum dilutions of 1/800 & 1/1600 were tested in the assay although this did result in a lowering of the analytic sensitivity.

Later stages of the assay validation included ELISA data from tests on 262 serum samples from known sero-positive populations of common and koi carp from 8 sites with a history of KHV disease (KHVD) and from tests on 475 serum samples from sero-negative populations of carp from 20 sites with no history of KHVD. Also included was data from tests on 72 serum samples from 3 sites where a high prevalence of carp pox was observed in the common carp population. This data was then used to establish a more robust positive negative cut-off or threshold.

The use of the sample to positive (S/P) ratio rather than a specific OD value, to determine cut-off values in the test, gave a significantly better overall diagnostic performance. A high positive/negative cut-off was set at a S/P ratio of 13.67% and this gave a diagnostic specificity (D-SP) of 98% and diagnostic sensitivity (D-SN) of 72%. To increase diagnostic sensitivity a low positive/negative cut-off was set at 6.02% and this increased the D-SN to 80% and gave a lower D-SP of 91%. Although the ELISA has only a moderate sensitivity (D-SN 72-80%) the performance characteristics showed that the assay was effective at the population level with a cut-off set to give a specificity of 98% or higher. The assay is not as effective as a tool to determine the exposure of individual fish.

Minutes

Sera from experimentally infected carps were used to develop an ELISA method for detection of KHV. The development has required optimisation at several levels e.g. the dilution of sera. In general the method can effectively be used on a population level whereas it is not that effective to use on individual fish. If the method is used in other laboratories, it may require further optimisation – especially where laboratories works at higher temperatures to ensure high robustness of the method.

Questions

Giuseppe Bovo: Does the temperature affect the results of the ELISA as can happen for e.g. SVCV?
***Keith Way*:** Antibody has been detected in sera sampled in different months – the best season to sample is when temperature increases – see paper in JFD + paper in press on distribution of KHV.
***Sven Bergmann*:** Is it the N-gene that is recognised by the antibodies and what about antibody kinetics – at what time does the antibody arrive in the serum?
Keith Way: Whole viruses have been used in the assays. Concerning the kinetics, the cut off dilution is relatively high. After about 3 weeks you should see antibodies.
***Niels Jørgen Olesen*:** How much purified virus did you use in such assays?

***Keith Way*:** We grow the virus on CCB cells and generate enough antigen for 6 months from 12 (75 cm^2) flasks.
***Sven Bergmann*:** KF-1 cells over passage 100 do not grow virus well.
***Keith Way*:** We have made the same observation but we have not seen that so far with CCB cells.

Development of serological methods for detection of Koi herpes virus (KHV) antibodies in carp*, Cyprinus carpio*

J. Castric*, N. J. Olesen, S. Bergmann, G. Bovo, O. Haenen, E. Jansson, M. Matras, D. Hongan
Afssa Ploufragan/Plouzané, Unité de pathologie virale des poisons, France

Abstract: Due to the difficulties encountered to isolate or identify CyHV-3 in asymptomatic carriers carp, a one year project on KHV serology has started between seven participants from European laboratories and one from China.
The main objective of this EPIZONE project is to develop, validate and implement serological techniques, seroneutralisation, immunofluorescence and ELISA, for detection of antibodies against KHV. The project will aim to compare the three methods regarding sensitivity, specificity and applicability under standard laboratory conditions. Different parameters will be assessed: viral strain, susceptible cell lines, antigen preparations, incubation conditions etc. The project has started in March 2009 by exchange of cells, virus, rabbit and carp sera between the partners. After the best cell line for virus production and titration has been chosen, the serological techniques will be adapted and tested so that standard operation procedures could be made available for all participants. An inter-laboratory proficiency test will then be organized in order to validate the different techniques and evaluate their applicability in the surveillance of carp populations regarding KHV.

Questions
***Olga Haenen*:** It is important to have more methods for KHV diagnostics and to have a method where you don't have to kill the fish.
***Francois Lieffrig*:** In grass carp, are conditions the same?
***Jeanette Castric*:** In general yes.
Keith Way: There is no evidence that KHV replicates in grass carp.
***Rob Raynard*:** Keith Way have already a good ELISA method quite similar to what you are developing - is this project repetition of previous work? Do CEFAS have available some of the reagents already?
***Keith Way*:** All of the reagents apart from the coating antigen of purified KHV are commercially available.
Jeanette Castric: When we started this project the publication was not available and the project was started in Epizone regi.
***Niels Jørgen Olesen*:** There should be collaboration between us and CEFAS. One advantage with more tests as SNT, ELISA and IFAT is that you can compare the tests. Until now CEFAS' work has provided a commercial test that is not publicly available. We would like to develope a test that is publicly available.

Case story from Giuseppe Bovo:
Presentation of surveillance for and detection of IHN Infection. In this case one sample (surveillance) was tested positive by PCR. However, there was no virus isolation and no mortality.

The farm was put under restriction - continued surveillance. Three months later: very few fish showed clinical signs. Sampling was made on symptomatic and non symptomatic fish. No virus positivity has been detected. When we went to serology (50% PNT) we observed 20% positivity in symptomatic fish (1/5) and 41.8% in non symptomatic fish (23/55) These data suggest that in endemic or chronic situations serology could help a lot for a correct diagnosis results

Questions

***Giuseppe Bovo*:** Regarding the negative result from symptomatic fish, an explanation could be that some viruses have a high titer whereas others have a low. If the sample furthermore contain blood – maybe neutralisation in vivo occur?

***Niels Jørgen Olesen*:** Maybe we should look more into serology for surveillance?

***Brit Hjeltnes*:** Were the samples PCR negative?

Giuseppe Bovo: Yes, only the first test was PCR positive. However, the sensitivity of this procedure is low – as stated by the OIE reference laboratory and is mainly used for identification after virus isolation.

Birgit Oidtmann: It is a general questions mark for testing systems if the isolation did not work.

***Giuseppe Bovo*:** It is problematic for IHNV – Serology methods might help.

Brit Hjeltnes: For surveillance – do we have proper tests?

***Niels Jørgen Olesen*:** Serology is most valuable in endemic areas. So we have to look for the purpose of the test.

MINI-WORKSHOP on implementation of Council Directive 2006/88/EC

The Key issues to be addressed when implementing the Council Directive 2006/88/EC

Sigrid Cabot
European Commission, Health & Consumer Protection Directorate-General, Unit D1 - Animal health and Standing Committees

Abstract: Council Directive 2006/88/EC lay down animal health requirements for aquaculture animals and products thereof and contain provisions on the prevention and control of certain diseases in aquatic animals.

The provisions of the Directive can be divided into three elements/pillars:

(a) the animal health requirements to be applied for the placing on the market, the importation and the transit of aquaculture animals and products thereof;

(b) minimum preventive measures aimed at increasing the awareness and preparedness of the competent authorities, aquaculture production business operators and others related to this industry, for diseases in aquaculture animals; and

(c) minimum control measures to be applied in the event of a suspicion of, or an outbreak of certain diseases in aquatic animals.

Compared to the previous legislation on aquatic animal health (Council Directives 91/67/EEC, 95/53/EEC and 95/70/EC) the following elements are either new or strengthened and thus would need special attention in the implementation of the Directive:

- The authorisation of all farms and certain processing establishments

- The establishment of a farm register
- Stronger focus on preventive measures, including risk-based animal health surveillance of all farms and traceability.
- Broader disease notification requirements
- Strengthen contingency plan requirements for emerging and exotic diseases
- New listed diseases: (KHV, EUS and EHN)

Minutes

Scope of the directive:

-Placing on the market, movement, import and transit of aquaculture animals or products thereof

-Minimum preventive measures for awareness and preparedness of diseases

-Minimum control measures in the event of suspicion of, or outbreak of certain diseases.

Aquaculture businesses has to be authorized, so does processing establishments involved in disease control – derogations from this: Animals that are kept without intention to be put on market, put & take, and businesses that will only sell small quantities directly to final consumer or local retail establishments directly supplying the final consumer.

When applying derogation: The directive shall apply mutatis mutandis: take into account the nature, characteristics and situations of the installation in question, and the risk of spreading disease as a result of its operation.

Conditions for authorization; Traceability (keeping of records of movements and mortalities), good hygiene practice, animal health surveillance scheme. Once the authorization is given, controls should be carried out.

Member states shall make a publicly available list of authorized aquaculture production businesses (APB). Internet based information page – decision 2008/392. This is a way to facilitate trade between farmers in different countries. This has to be put in place before August 1st 2009.

Risk based surveillance scheme: All farms and mollusc farming areas. Can be done by competent authorities (CA) or qualified aquatic animal health service. What to do: Advice farmers on animal health issues. Detect mortality and listed diseases. Sampling and laboratory analysis not obligatory but to be decided at inspection. How often the farm should be visited depends on the disease category within which it is listed, and the risk level of the farm (Decision 2008/896/EC – guidelines).

Disease notification: Early warning system; notification to CA of increased mortality or suspicion on listed disease. Notification through ADNS (decision 2008/650/EC)

Disease control

Listed exotic diseases: Import requirements for susceptible species and vectors. Eradication if detected in community.

Listed non-exotic diseases: Suspicion shall be notified. Part of general surveillance. If detected: eradicate or contain, according to disease category and national disease strategy. Disease specific placing on the market requirements.

4 ways to achieve disease freedom:

1. No susceptible species
2. Pathogen can not survive in the area
3. Historical freedom
4. Targeted surveillance

National measures: Member states may take appropriate and necessary measures to prevent the introduction or to control other non-listed diseases, which pose a significant risk. Measures that affect trade between Member States must be approved by the Commission under the Comitology procedure.

NRL
Legislation implementing directive 2006/88/EC: 1251/2008, 1250/2008, 2008/946.

Sampling and diagnostic plans on VHS, IHN, KHV and ISA

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Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell'Università 10, 35020 Legnaro (PD) Italy.

Abstract: Following the publication of directive 2006/88/EC and because of the experience reached during the last years there is an urgent need to improve the existing legislation concerning sampling methods, surveillance programmes and diagnostic procedures to be adopted both for old and new listed diseases.
No official documents has up to now been issued by the Commission and this presentation is tightly based on the opinions and suggestions of a group of invited private/governmental experts and in any circumstances may be regarded as stating an official position of the European Commission. Contrary to the previous decisions no detailed laboratory procedures will be included in the next manual which will contain only general information while the complete details will be available at the CRL web-site. Besides to basic requirements already introduced in the previous manuals, like the species to be sampled, tissues to be processed, sampling season, interval between two consecutive controls, additional criteria will be introduced as the minimum diagnostic requirements to rule out the infection suspicion or the possibility for immediate reinstatement of the free status.
Different surveillance programmes, to be adopted according to the actual status of compartments/zones will permit to achieve the free status in 2-4 years. In addition the possibility for immediate reinstatement of the free status will also be introduced in the new legislation, only applicable to previously free individual farms which free status has been withdrawn and provided that the sanitary status is independent from surrounding natural waters and if the epizootic investigation concludes that the disease has no further spread to other farms or in the wild.
In some instances, as in the case of KHV, the surveillance plans will be influenced by the density of carp farms inside a catchment basin and when their number is considered limited and targeted surveillance does not provide sufficient epidemiological data of the whole basin sampling would necessary include fish from the wild.

Minutes
The next diagnostic manual will contain sampling details, surveillance programmes, criteria for disease suspicion and confirmation and diagnostic methods. This manual will be available at the CRL website.
Containment areas are defined based on a case to case analysis. The containment area includes the production zone and a 10 km radius surveillance zone.
Sampling criteria set up for VHS & IHN according to the specific disease.
Model A: 2 year surveillance scheme
Model B: Immediate reinstatement of disease free status (previously free, independent from other farms)

Model C: 4 year surveillance programme with reduced sample size.
In order to maintain disease free status, the farms must be in a surveillance programme where sampling is done according to risk level of each farm.

A model for risk ranking fish farms to inform disease risk-based surveillance

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Abstract: Until recently, fish farms involved in disease surveillance programmes in the EU have been visited and samples collected following a prescribed, non-risk based approach. The recent European Council Directive 2006/88/EC on aquatic animal health requires that risk-based animal health surveillance is applied to each aquaculture production business (APB) in the EU. The frequency of visits should take account of the likelihood that a fish farm may contract and spread disease and this requires an assessment of the level of risk applying to each APB. To assist in this process we have developed a model for risk ranking fish farms that can also be applied to other types of APBs (e.g. mollusc farming areas, crustacean farms).

Through stakeholder consultation and input by aquatic animal disease experts and epidemiologists, we have identified a range of risk factor themes: (1) Live fish movements on and off site, (2) Exposure via water, (3) On site processing, (4) Biosecurity, (5) Management practices, (6) Geographical factors, (7) Mechanical transmission and (8) Other routes. We demonstrate how information regarding these risk factors can be assessed and combined to achieve risk scores for introduction and spread.

Minutes

Farms can have different categories for the different diseases.
The presented model is for salmonid farms, with regards to VHS – focus was category 1 farms, using a semi-quantitative approach.

Risk factors: live fish movements, exposure via water on site processing, biosecurity, management practices, geographical factors, mechanical transmission, other routes/ risk

Quantitative factors: Live-fish movements, likelihood of farms to become infected/spread disease within a year.
Semi-quantitative factors: Exposure/spread via water, hazards, flooding. Give a score instead of numbers.

Example: Likelihood of farm becoming infected from live-fish movement: probability of source being infected (number of breakdowns of disease-free farms out of all free farms) x number of sources.

The model is transparent and allows the farmers to see how their score was calculated and how any of their activities influences the score.

Birgit has CD's with the programme for the model.

Workshop on the implementation of the new Fish Health Directive – group Northern Europe
Chair: Birgit Oidtmann. Reporter: Birgit Oidtmann.

Questions discussed:
Have member states completed the process of authorising and registering Aquaculture Production Businesses?
Which types of APBs were authorised opposed to registered?
Have they risk ranked their farms?
Have MS categorised all their farms with regards to their disease category?
For member states that have farms that fall into Cat. III, do they intend to move out of this category, or do they consider Cat. III as a permanent status?

Most MS were well progressed with regards to authorising and registering farms.

- The Faroe Islands have 6 salmonid APBs and 4 processing sites that are authorised. Transporters were authorised and so were the 4 or 5 put and take fisheries in their country.
- England &Wales have about 580 finfish APBs that were authorised. Furthermore, there are about 60 mollusc farming areas and 52 purification centres. There is currently no authorised processing plant. About 2000 fisheries are currently registered, although this does probably not comprise all fisheries yet that exist.
- Denmark has around 345 fin fish farms, 15 processors, 65 mollusc farming areas, and 10 crayfish farms – all authorised.
- Ireland has 99 fish farms, 3 processing plants and 450-500 mollusc farming areas. The authorisation of finfish farms and processing plants is completed. The authorisation of the mollusc farming areas is still in process. Ireland will authorise put and take fisheries. Transporters will be registered.
- Sweden has 140 fin fish farms that will all be authorised. A decision regarding the classification of crayfish farms has not yet been made. The 6 mollusc farming areas will be authorised. The number of processing plants is currently unclear. Transporters will be registered. Sweden has around 300-400 put and take fisheries.
- Finland had to make legal amendments to implement the directive. This was needed in order to have the legal basis to request authorisation of aquaculture farms and processing plants; publish information of the authorised farms/companies on the web
  Finland has around 500 fin fish farms (150 marine, and 350 inland) and 50 crayfish farms. About 80% of fish farming companies have submitted their application for authorisation to the Competent Authority and only 7 of the 50 crayfish farms. Therefore some follow up is still required.
  A threshold by production was set to decide whether APBs were registered or required authorisation. The threshold is 2,000 kg/year for fish farms or less than 2,000 kg of fish feed purchased by the farm and fish only being sold locally.
  Crayfish farms did not require authorisation if they produce less than 2000 animals/year and sell only locally. Derogation from authorisation also applies to angling ponds stocked with farmed fish, shellfish farms (reared for cleaning water) ornamental fish farms not in direct contact with the water systems.
- In Norway, all fish farms have been authorised. Put and take fisheries don't exist.
- In Scotland, the authorisation is still in process. Fisheries have not yet been registered.

Risk ranking of farms and surveillance programme:
Most MS have risk ranked their farms and worked out their surveillance programmes. The surveillance programmes are above the minimum requirements presented by

Guiseppe Bovo earlier that day.

- Ireland has completed the process of risk categorising its farms. Farms were ranked depending on production type. Ireland has decided on 2 visits/year for high risk farms (1 Art. 7, 1 Art. 10), 1 for medium risk farms (alternating in 1 year Art. 7 visit, in next year, Art. 10 visit). Low risk farms will be visited every other year.
- Sweden is still in the process of risk ranking its APBs. Farms are currently grouped by criteria such as size, location, and whether or not they are restocking, but risk ranking criteria to be used still need to be finalised, but focus will be laid on live fish movements.
- Scotland has completed the process of risk ranking its farms.
- Denmark has completed the process of risk ranking its farm during the eradication program for VHS.
- Finland has decided on its surveillance programme and set criteria for increased sampling frequency; these include: live fish movements from the marine to freshwater farms, imports of live eggs from abroad, marine farms located in the vicinity of slaughter houses, and others.

Categorising farms into Cat. I-V

This process was largely completed for all salmonid farms. Most salmonid farms in Northern Europe would have had a status prior to the implementation with regards to VHS/IHN. Therefore, this could easily be transposed into the new FHD.
With regards to cyprinid farms: some countries, such as Norway, don't have cyprinid farms and therefore no need to categorise them. In those countries, that do have cyprinid APBs, virtually all farms are currently in Cat. III with regards to KHV.
There was insufficient time to discuss how this has progressed for crustacean and mollusc farms.

Status of marine RBT farms

The question of how to deal with marine RBT farms was discussed. Niels Jørgen Olesen had earlier mentioned that Denmark had decided to place marine RBT farms into Cat. III. This was based on the assessment that, given the continuous exposure of the fish to VHSV from wild fish, it seemed not reasonable to class these RBT farms as Cat. I. Large RBT may be used for freshwater fisheries, which could jeopardise the status of freshwater sites. It seemed not sensible to put freshwater sites at risk by granting marine RBT sites Cat. I status.

It was felt that if fish farms that are at a high risk of becoming infected, such as the example of a RBT farm in the marine, would obtain Cat. I status, farms of Cat. I status receiving fish from such sources would equally be under a possibly constant threat of becoming infected. Therefore, it was questioned whether farms currently free from a listed pathogen should ever be given Cat. I status, given that these farms may pose a high risk of becoming infected. The consequences for an importing country could be substantial, e.g. a member state might loose its disease free status. Therefore, it was questionable, whether Cat. I status can be granted to APBs free from infection, but at a high risk of becoming infected.

On the question of how to deal with

Duration of Cat. III status:

Different views were expressed on whether or not farms should be allowed to stay for an extended time period in Cat. III.
Scotland felt that farms should be allowed to stay in this category, whereas Ireland took the view that Cat. III was supposed to be a transitional category.

Workshop on the implementation of the new Fish Health Directive – group Continental Europe

Chair: Niels Jørgen Olesen. Reporter: Olga Haenen.

Czech Republic: Farms are under national survey program for VHS, IHN and IPN, and also some for KHV. There are no approved farms according to EC legislation, and the program has not yet been sent to the EC.

Estonia: On historical basis they are Cat. I. Representative was not sure, if the application was sent already to the EC.

Austria: Data are mainly focused on VHS & IHN national program. Since 2006 also KHV and IPN testing. Most facilities are in group III. There is not yet surveillance according to the EC for category III, but there is for cat. II. When Cat. I would be reached is not sure yet. There is only 1 farm in cat. IV, so no cat. V at all, i.e. no infected farms.

Germany: Many farms in cat. I in non approved zones, some in approved zones. No clue about the missing farms in the scheme, the high number is probably due to the high number of non-registered carp farms. Still approx. 2000 rainbow trout farms are missing from the lists? Sven Bergmann: Probably problem with the registration of farms, as there are so many. Also it is unclear whether not-authorized but registered farms shall be categorized. Heike Schütze: 2nd problem: many hobby farms? Where to put the border yes/no registration? Some federal states have governmental service (vet.), but some are private. 12 Farms in cat. V is a too low number, as there were >6 outbreaks of IHN lately already. Sven Bergmann will meet the ministry next week and will ask for clarification. Also the 0 farms for KHV in cat. V is nonsense. Problem: FLI is not involved in the data for this table. Sigrid Cabot: The only application the EC received from the whole EU with a proposed control program on KHV is from Saxony. Category IV is 40x carp farm, according to Sigrid.

Belgium: The directive is not yet implemented in the federal system. Twice a year 30 fish from S-Belgium are under survey, and 3 salmonid farms in S-Belgium are infected (cat. V). The carp production is mainly in the north. In the lab, KHV is often diagnosed in koi, but the sources are unknown (closed/open?). The health situation has improved in S-Belgium. The survey program is not yet approved by the EC.

Netherlands: Niels Jørgen Olesen remarks, that all salmonid farms of the whole EU should be in cat. I for ISA, as the EU is free of that disease. Olga remarks, the missing farms are farms with non-susceptible species. It is confirmed by Sigrid, that only susceptible species should be categorized.

France: Data came from the authorities; Jeannette Castric is surprised that no cat. V farms were given for both IHN and VHS! Cat. I is true, right values. Cat. II: true too, they will move to Cat. I in 2 years. Brittany, Charentes and S-W France are free of VHS and IHN. There is only a surveillance program for salmonids. The KHV data might be right. Cat. I and II are all registered, small farms are difficult to register, and this will not be ready before the end of July 2009. There are problems with risk evaluation, working on data surveillance before submitting the plans to the EC.

Sigrid Cabot, in general:

- The end of June a meeting is foreseen to see, how far the MS are with their implementation.
- The MS will be reminded to have their registers in place, according to EC/2009/177.
- Many data which are presented today are not known yet to the Commission.
- Sigrid explains the trade possibilities between categories, as given in the directive.

Switzerland: The 5 farms in Cat. I and II are regularly tested, but this is not yet approved by the EC. Thomas Wahli doesn't think he could get that approval. Switzerland has: 1) a program to register all farms, and 2) a project to develop a strategy for a risk based surveillance program in accordance of EU regulations. Both have just started.

Lithuania: In April 2008 the implementation was started, and now 28 farms are registered. The data came from the Veterinary Service, so the delegate have no details.

Hungaria: There is a survey program for SVC since 1999-2008. No virus could be found (SVC and other viruses, except iridoviruses). In 2009 an EHN-program has started. For KHV, 120 farms was tested by real time PCR, and they were all negative. There are 1 or 2 surveys per year. The application for disease free status was submitted to the EC by Sept 2008, for KHVD. Hungaria has a risk because of geography, especially for KHV. All carp farms are tested (consumption- and production farms). All lakes should be tested still for KHV.

Latvia: The state surveillance program includes fewer farms (totally 30) than given in the table. For KHV testing there are 20 farms, which produce carp and other species, but for salmonid diseases surveillance - 16 farms, which produce salmonids (farms and state hatcheries, which produce carp, salmonids and/or other freshwater and marine species are included in both surveillances). Small hobby fish farms (totally 143) are not included in surveillance program. The program is not yet approved by the EC. Sigrid Cabot: Yes, the farms should be in cat. III, but only after EC approval they can move. Latvia: The state wants 2 registers: 1) state hatcheries & farms with surveillance, and 2) hobby fish farms. Not sure if that is a good idea.

Slovakia: All cat. I farms are registered and under surveillance. In 2008 there were 2 outbreaks of VHS, and another outbreak last week. A program has been made by the veterinary authority to eradicate the disease. It is not known if we can get cat. I based on historical grounds or only on monitoring. If approved by the EC we can adapt the data.

In general:
Sigrid Cabot: The application is not very complex, and you will soon get an answer from the Commission. Niels Jørgen Olesen: There are many cat. I farms now, so this will influence the trade. Sven Bergmann: You are only cat. I when officially approved by the EC. Niels Jørgen Olesen: Yes, only an official sampling & frequency will be authorized, not many countries will have this already in place.

Poland: Nearly all rainbow trout farms are surveyed, but not officially yet. Some of the cat. V KHVD farms should be Cat. III. Niels Jørgen Olesen: Yes, when you have them all in cat. V, all fish may enter Poland! Sven Bergmann: And when you are cat. V you can only trade with cat. V, whereas there is a lot of trade between Germany, Czech Republic and Poland.

Luxembourg: François Lieffrig tells there are no data, but there are 2 farms under survey by Belgium.

General points for discussion
- Olga Haenen, NL: Our veterinary service has problems with the practice of certificates, especially the import health certificates.
- Tomáš Veselý, CZ: we need 1 system, transparent for all MS.
- Sigrid Cabot, EC: Zones of >75% of the area of the MS are o.k., and smaller sites should be on the website. It is good to see today, that there are many national programs in place.

- Niels Jørgen Olesen, DK: The implementation and zoning should be coordinated through your CVO.
- Sigrid Cabot, EC: If the CRL publishes these data, there should be a disclaimer, because the data may not be correct. It may be misused for trade namely. Take care!
- Niels Jørgen Olesen, DK: What about registration in MS? Tomáš Veselý, CZ: There are hundreds of authorizations still in process, but it goes o.k., including geographical coordinates. Sven Bergmann, GE: The regional fish health services add coordinates in some federal states, in others not. Niels Jørgen Olesen: The coordinates are not obligatory to add, but could be useful. Is the federal law in Germany blocking the EU law? Sigrid Cabot: This should not be the case, as we just need information of business, and not on personal data, it is a community law, so approved by all MS, and it should be followed, the national privacy regulations are overruled by the EC. Sven Bergmann: will ask his government on this the 9th of June 2009.

Workshop on the implementation of the new Fish Health Directive – group Mediterranean Europe

Chair: Giuseppe Bovo. Reporter: Giuseppe Bovo.

Italy: Different registration has been used in some regions. So the first challenge is to unify the coding. The CA is building a database in order to compile this information. The database should be finished by summertime. There has been no categorisation yet. The ministry wants to be declared disease free in to category 1 immediately. Marine farms are mainly rearing non susceptible species like sea bass or sea bream; they will be put in category 1. Put and take fisheries connected with the river system will be considered as farms, registered, authorised and categorised. Those put & take lakes that are not in contact with waterways will not be authorised and categorized, but only registered. They are never visited. The problem with put & take facilities is that they have no interest in getting rid of disease, so they are always infected. It is very good that this directive requires that all farms be registered. Now we can have en exact view of how many farms there are, and their health status. The ministry is not happy about making it public.

Turkey: There is no implementation of this directive, unfortunately. Probably should do so in the future, because of the trade with EU-countries.

Spain: Is similar to Italy. Have a database for fish production units, and is preparing a new database with data on the farms and including their health status to be used for the categorisation. These databases are connected and will be made public. The owner of the database is the Ministry of Agriculture, but every region has their own database that is connected to this one. The registration is in the regions, so it depends on which region.

Romania: Some farms are authorized but within an old system. For the new authorization, we are waiting for the new draft to be approved. There is a register of the farms, and this has to be transferred to the competent authorities.

Serbia: Implementation is at the beginning: the fish farms are centrally registered, in department of agriculture, but it has not been made public yet. The farms have not been categorized. There is a programme for certain diseases; SVC, IPN, VHS and a few bacterial diseases. So there is some data about the health status of some of the fish farms.

Bosnia-Hercegovina: For the last 5 years there has been a surveillance programme. There are 28 salmonid fish farms and 5 carp farms that are centrally registered by the veterinary authorities –

they have a veterinary number and are under inspection. In total there is about 120 salmonid farms, 25 cyprinid farms and 2 marine fish farms, but only the previously mentioned are veterinary registered, whereas the others are registered, but not by the veterinary service. They have officially never been visited by the veterinary services. This year monitoring is started on two diseases. Most farms will be included in category II.

Slovenia: Is trying to follow the deadlines. Authorization is in progress, 22 farms are authorized according to the rules of the directive. The majority of farms have been registered with regard to species, trade and so on. We are mostly acquainted with the health status, since there has been surveillance for many years. All fish health management is centralised. We are finishing the application of the website - making the list public before the 1st of August - it is being tested now. Fish farmers are not interested in the surveillance by the Commission Decision. At the moment there are three farms in the category I - you can find them on the website, already. However we have our own surveillance programme paid by VARS (Veterinary Administration Republic of Slovenia). Regarding this programme all hatcheries and fish farms with life fish market are monitored for VHSV and IHNV. Other farms are tested regarding the clinical and pathoanatomical signs presumptive to VHS and IHN or regarding to the epizootiological investigation. Unfortunately fish farms in "our programme" correspond to the category III. In all others the health situation is unknown and is officially infected. In fact we have got 3 VHSV infected fish farms. We are encouraging fish farmers for its eradication. The working team for fish diseases management has prepared a manual for good hygienic measures, and designed a questionnaire for risk ranking.

Portugal: All farms are in category II, because they are not declared disease free, but there has been a surveillance programme for 15 years. Plan to apply for historical freedom as documentation for placing in category I. The directive is still not implemented in Portuguese legislation, but we are preparing for authorization and categorization. Two fish farms are just now moved to category I.

Bulgaria: The directive has been published in the state gazette. All farms have been registered, and geographical details are also under way. 47 trout farms, 199 carps, 4 crustacean farms. In the register is name, address, type of production, details on water supply. Work is undergoing to include health status and categorisation of farms. Surveillance programme was initiated 3 years ago. 24 trout farms are in category I for VHS, 1 farm is in category IV and one in category V. We intend to implement passive surveillance for KHV.

Greece: The Directive was implemented in national legislation in March 2009. All farms, including marine farms, will be registered before end of July. What happens if someone has sites in both Greece and Turkey? Trout farms and cyprinids will be in category III. The ministry takes care of surveillance.

Cyprus: All the farms (20 in total) are registered. The Directive has been implemented. There is 6 farms with rainbow trout and they are in category I for VHS and EHN. 2 farms with Koi carp are in category 3. Authorization is by veterinary authorization.

Croatia: The legislation is in place. All farms are registered. There is a national surveillance programme for listed diseases including SVC. Farms are not authorized; therefore all farms are put in category III. Internet-site is under preparation. Database with geographic positions is being prepared.

Albania: The directive is not implemented yet, but there is surveillance of farms. The registration is now under Ministry for Environment, but needs to be under VA.

Kosovo: The directive has not been implemented in local legislation yet. There are 50 farms.

There is confusion what is meant with "farms that provide only for human consumption" – Does that mean that this is facilities that only buy living fish and stock them for a few days and then sell them for human consumption? So no "farming" as such, but only stocking?

Until the farms are authorized, they cannot be categorized. So many of the farms that have been noted to be in categories in the questionnaire are based on previous legislation.
Need manual on good hygiene practice and the demonstration that there is a surveillance programme, before farms can be authorized.

In many countries the registration is in different departments, and this has to be coordinated.
First of all; all farms should be registered, then they should be authorized, and then they can be categorized. A lot of work: surveillance schemes, hygienic manual etc.

Workshop on the implementation of the new Fish Health Directive – summary of group discussions

Northern European countries: Authorization: DK, Faeroe Islands and Norway are finished, and rest will finish before summer. All have surveillance programmes in place. There has been discussion about susceptible species. Should those that are not susceptible still be categorized? This does not make sense, only relevant diseases and species should be categorized. Risk-ranking: Denmark, and UK has already done it. The focus has clearly been on trade with live fish.

Continental European countries: There are big differences in the way the member states have categorized the farms. It is important that this is harmonized. Some countries are registered free of KHV, but it is uncertain if this is approved by the commission. In some of the states it is not allowed to put private details like name and address on the internet, but since this is EU legislation, they will have to comply.

Mediterranean countries: In some countries the Directive has not yet been implemented in local legislation. Often the farms are registered in one ministry and have to be transferred to the veterinary authorities. Some countries have several regions that each has their own codes, and it is difficult to harmonise this. Remember that even if the farms have no susceptible species, they still need to be authorized and surveyed every four years.

SESSION III Scientific research update

Susceptible species to listed diseases - EFSA Report

Ana Afonso* and Franck Berthe
EFSA Animal Health and Welfare Panel

Abstract: Following a request from the European Commission, the Panel on Animal Health and Welfare was asked to deliver a scientific opinion on aquatic animal species susceptible to the diseases listed in the Council Directive 2006/88/EC. More specifically, the question was to establish i) which species other than those listed in Part II of Annex IV to Directive 2006/88/EC that could be considered as susceptible and ii) which of the species currently listed as susceptible in Part II of Annex IV to Directive 2006/88/EC cannot be considered as susceptible.

A comprehensive literature review was performed with considerations for: i) reflection of natural pathways provided by the experimental design of reported studies, ii) compliance with four objective criteria pertaining to susceptibility to infection, and iii) thorough identification of the causative agent.

The four criteria used to assess susceptibility of host species were: evidence of replication or growth of the organism (A), presence of a viable organism (B), presence of specific clinicopathological changes (C), and specific location of the pathogen (D).

This led to identification of two main groups: Group I, host species for which the quality of the data provided clear support for susceptibility, and Group II, host species for which incomplete or unclear data prevented a clear conclusion or the only available data was obtained from invasive experiments. Group I (susceptible species) contains i) traded and non-traded species, ii) species belonging to several genera, and iii) many were susceptible to several of the specified pathogens, so may represent different levels of risk.

Within Group I, species were identified that currently are not listed in Directive 2006/88/EC and those species are recommended to be considered for possible inclusion. Partial evidence suggesting susceptibility was obtained for a large number of host species (Group II). Several host species, including some currently listed in Directive 2006/88/EC, were identified as potentially non-susceptible but it was not possible to confirm this status firmly due to the quality of the data.

Further scientific studies are required to resolve the uncertainty concerning the susceptibility of the host species identified in this group. Such studies should apply clear criteria, such as those used in this opinion, to assess susceptibility of host species and clear identification of the pathogen and affected host(s). In addition, the opinion noted that the lack of clear case definition for some of the specified pathogens compromised assessment of the susceptibility of some host species.

Minutes

The objective of this work was to revise the list of susceptible species in the annex of CD 2006/88/EC based on available scientific literature. A risk assessment approach was not followed. Neither was it taken into consideration whether the fish were farmed or imported into EU.
The question of susceptible species was raised by the vector report (for links to the reports, see http://www.crl-fish.eu/useful_links.aspx). Vectors = mechanical carriers.
A problem in the work has been that a clear case definition has not always existed, and the pathogen ID is not always clear, especially for mollusc pathogens.
The list should be reviewed regularly.

Questions

***Sven Bergman*:** Can you explain the difference between whitespot syndrome virus in decapods and KHV in cyprinids? Why are all decapods listed as susceptible for whitespot syndrome virus when all cyprinids are not for KHV?

***Ana Afonso*:** EFSA can not answer to that question since it is a risk managers decision.

***Birgit Oidtman*:** For whitespot syndrome virus, every decapod tested has been found susceptible. I am not sure if this is the case for cyprinids and KHV.

***Ana Afonso*:** The criteria of when to include the whole group or not should be clearly defined based on scientific principles.

***Niels Jørgen Olesen*:** How do you think the Directive will look like after this work? What is your recommendation?

***Ana Afonso*:** EFSA is created to give scientific advice, but the legislators should also take other things into account. It is up to the Commission to decide what to do with this report. The EFSA AHAW panel recommended that the species in group I should be listed as susceptible species, but a risk assessment may have to follow before taking new species into the list.

***Sven Bergman*:** There are no cyprinids which are not infectable and do not grow the virus.

***Olga Haenen*:** The report could only take into account peer reviewed literature, so hopefully the cyprinid-KHV work will be published soon and taken into account when the EFSA reviews it.

Establishment of a CRL-database for fish pathogenic viruses

Søren Peter Jonstrup*[1], Tanya Gray[2], Søren Kahns[1], Helle Frank Skall[1], Mike Snow[3] and Niels Jørgen Olesen[1]

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Abstract: A database has been created, www.FishPathogens.eu , with the aim of providing a single repository for collating important information on significant pathogens of aquaculture, relevant to their control and management. This database will be developed, maintained and managed as part of the European Community Reference Laboratory for Fish Diseases function. This concept has been initially developed for VHSV and will be extended in future to include information on other significant aquaculture pathogens. Information included for each isolate comprises sequence, geographic origin, host origin and useful key literature. Various search mechanisms make it easy to find specific groups of isolates. Search results can be presented in several different ways including table based, map based, and graph based outputs. When retrieving sequences, the user is given freedom to obtain data from any selected part of the genome of interest. The output of the sequence search can be readily retrieved as a FASTA file ready to be imported into a sequence alignment tool of choice, facilitating further molecular epidemiological study.

Minutes

We have chosen to use open source software, it is a low cost platform that everybody can access.

You can add virus reports and sequence reports separately, as it is not always the same persons doing the isolation and the sequencing.

Everybody can search the database. You can do map based search, blast based search or a text based search.

Manuals are available on the website.

A publication on the database is accepted and will be published soon in Journal of Fish Diseases.

In the future the database will be extended, first with the other rhabdoviruses, but also including e.g. KHV and ISAV.
At the moment there is app. 230 isolates, most of them are Danish. More isolates will be added in the future, and we invite everybody to add their isolates. If you need help for adding information please contact us, and we will guide you.
It is possible to add private notes that are not publically available, and in case you are not yet ready to release anything in the report yet, the whole report can have restricted assess.

Questions
***Irene Ørpetveit*:** Do you still wish people to publish in GenBank and like?
***Søren Peter Jonstrup*:** At the moment you are still required to publish in GenBank when publishing, but we hope in the future, that we can do this for you, when you put data into the CRL database.

Molecular characterization of VHSV and IHNV in Germany

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Abstract: The fish-pathogenic rhabdoviruses of Infectious Haematopoietic 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.
The identification of the viral glycoprotein gene sequence makes it possible to distinguish between the different isolates. The nucleotide sequences of the complete glycoprotein genes of German IHNV and VHSV isolates were determined and compared with isolates from other European countries. Comparative studies of the G gene sequences of IHNV and VHSV permit conclusions with regard to their origin, relationship and classification. Phylogenetic analyses illustrate the development and evolution of the viruses as well as the correlation between virus distribution and trade channel.
Main prerequisite for phylogenetic analyses is a close collaboration between the regional, national and international reference laboratories as well as the regional veterinary services. The European Union and the German government demand epidemiological studies of IHNV and VHSV outbreaks in free hatcheries. Until now, the identification and determination of the G gene are not subject to a regulation. In the last year 32 VHSV and 6 IHNV outbreaks were reported in Germany. Phylogenetic analyses were performed for 14 VHSV and 2 IHNV cases only. This situation is far from satisfactory. Therefore, the origin of an isolate often remains unclear. Using classical diagnostic methods a clear differentiation of identified IHNV and VHSV isolates is often impossible. Phylogenetic analyses permit a clear identification of isolates and their evolution as well as the tracing of trading practises.

Minutes
First I will start by bringing greetings from Peter Enzmann who has now retired.
IHNV consist of only 1 serogroup. Differentiation based on serology can only be done with MAbs. The original classification is based on the geographical origin of the isolates: RB (Round Butte; Salmon, Alaska and British Columbia), WRAC (Western Regional Aquaculture Center, freshwater

fish) and SRCV (Sacramento River Chinook Virus, salmon, California) corresponding to U, M and L genogroups.
VHSV, as IHNV, consist also of only 1 serogroup. There is no correlation between serology and pathogenicity.
We sequence the full length G-genes, and sequence a big chunk round the genes to be sure we have the correct G-gene sequence.
Phylogenetic analysis show influence/evolution of Swiss and Danish VHSV isolates and of French, Italian and Swiss IHNV isolates in Germany. The trade plays a big role in the distribution of VHSV in Germany.
In order to improve the epidemiological analysis we need good international cooperation and Gentleman agreements. A common database is a very good idea.

Questions
Very clear talk, no questions.

Distinction between genotypes of Viral Haemorrhagic Septicaemia virus (VHSV) using monoclonal antibodies

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Abstract: VHSV isolates can be divided into 4 major genotypes and a number of subtypes with an almost distinct geographical distribution. Host range and pathogenicity appear to some extent to be linked with genotypes. If once new genotypes of VHSV are introduced into new areas, they can cause severe outbreaks of VHS among susceptible fishes. According to the OIE Aquatic Animal Health Code, even if the same disease agent is present in both the import and the export country, the importing country can demand health certificate of the exporting country for imports when the pathogenicity or host range of the strain in the exporting country is significantly higher or larger than that in the importing country. In order to prevent introduction to or spreading in a country of new genotypes of VHSV and to facilitate the responsibilities of exporting and importing countries, such as issuing health certificates and carry out quarantine and disease control programs, a quick and simple detection method for discriminating between each of the genotypes of VHSV is strongly desired. Monoclonal antibodies (MAbs) VHS-10 and VHS-5.18 specifically recognizing VHSV genotypes IVa and Ib respectively, as well as MAb IP5B11 recognizing all known VHSV isolates, were prepared earlier. In the present study, additional new genotype specific monoclonal antibodies against VHSV were produced, aiming at establishing a complete immunoassay for typing of VHSV according to genotype.

BALb-c mice were immunized with purified preparations of 7 different genotypes (I, Ia, Ib, II, III, IVa and IVb). Six MAbs from these hybridoma clones were selected and their reactivity in IFAT and ELISA tested against a large panel of 79 VHSV isolates. The isolates represent all known geno- and subgenotypes of VHSV.

Among the new MAbs, VHS-1.24, reacted with all types except genotype Ie (the Black Sea variant of VHSV), while MAb VHS-9.23 reacted with all genotypes except genotype III. MAb VHS-3.80 reacted with genotypes Ib, Ic, Id and II, only. MAb VHS-7.57 reacted with genotype II and IVa. Interestingly, MAb VHS-3.75 reacted with all genotype III isolates except the rainbow trout pathogenic isolate from Norway (NO-2007-50-385) (Dale et al. in press), but did react with the New Brunswick VHSV IVb isolate (Oliver 2002, Gagné et al. 2007). Another MAb (VHS-1.88) reacted with genotype IVb only, except with the New Brunswick isolate. The present findings

support a phenotypic difference between NO-2007-50-385 and the other virus representatives in genotype III, and genotype IVb may eventually be split up in two subgroups (the Great Lakes isolates and New Brunswick isolate).

In conclusion, we can now distinguish between all genotypes and some of subtypes of VHSV by testing isolates in IFAT or ELISA with 9 MAbs (Table 1).

Table 1 Reactivity of MAbs for various VHSV genotypes

	Genotype of VHSV								
MAbs	I /Ia	Ib	Ic/Id	Ie	II	III	IVa	IVb-G.L.	IVb-N.B.
IP5B11	+	+	+	+	+	+	+	+	+
VHS-1.24	+	+	+	-	+	+	+	+	+
VHS-9.23	+	+	+	+	+	-	+	+	+
VHS-3.80	-	+	+	-	+	-	-	-	-
VHS-7.57	-	-	-	-	+	-	+	-	-
VHS-5.18	-	+	-	-	-	-	-	-	-
VHS-3.75*	-	-	-	-	-	+	-	-	+
VHS-10	-	-	-	-	-	-	+	-	-
VHS-1.88	-	-	-	-	-	-	-	+	-

+;positive
-;negative
*; The rainbow trout pathogenic genotype III isolate is not reacting.
IVb-G.L.: Genotype IVb , the Greart Lakes isolates
IVb-N.B.: Genotype IVb, New Brunswick isolate

Questions

***Søren Peter Jonstrup*:** Do you think the MAbs recognize a 3D structure and do you think they can discriminate between pathogenicity towards certain species?

***Takafumi Ito*:** I think they recognize a 3D structure, e.g. VHS-10 don't react neither on reduced or non-reduced conditions.

***Mike Snow*:** Are the MAbs neutralizing?

***Takafumi Ito*:** No, they are not neutralizing. Maybe because the epitope is not related to the G-gene. When doing IFAT it does not look like they react with G-proteins.

Status of the RANA-project

Britt Bang Jensen
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Abstract: The high pathogenicity of ranaviruses to fish and amphibians in both the EU and especially also in Australia and the USA have lead to speculations that these ranaviruses might pose a serious threat to both farmed and wild-living freshwater fish and amphibians within the European community. Therefore, the EU-project "Risk assessment of new and emerging systemic iridoviral diseases for European fish and aquatic ecosystems" (RANA, SSPE-CT-2005-006459) was initiated in June 2006 under the 7th framework programme. The RANA-project was carried out by six member institutions in the Czech Republic, Denmark, Finland, Germany, Italy and the United Kingdom and was finalised in February 2009.

The project has focused on the following topics:

-Developing and validating reliable diagnostic methods for identification of ranaviruses

-Establishing host spectrum and pathogenicity of ranaviruses in farmed and wild European fish and amphibians through experimental challenges

-Investigating the occurrence of ranaviruses in European amphibian populations and in imported ornamental fish

-Characterisation and differentiation of different ranaviruses by molecular methods

The project has generated a lot of results with regards to these topics, results which are currently becoming available to the scientific community via publication in peer-reviewed journals and presentations at international conferences.
The final outcome of the RANA-project has been a preliminary risk assessment on the risk of introduction and spread of exotic ranaviruses into the EU plus an extended and a detailed list of recommendations for the European Commission.

Minutes
EHNV, ECV and ESV can infect wild fish, farmed fish, ornamental fish and amphibians.
Both ornamental fish and farmed or wild fish could be infected with amphibian viral isolates.
Holopainen et al. have developed primers that show a very high degree of similarity between EHNV, ECV and ESV – these are 99% similar – and can infect similar fish species.
The RANA-project has looked into validation of detection methods. Giuseppe Bovo tested IHC protocols for identification of Rana viruses and found no discrimination on the different isolates besides doctorfish virus (DFV) and guppy virus 6 (GV6), which are different from the other ranaviruses. One purpose was to survey for presence of RANA viruses within the EU. In a project, 150 samples = imported frogs from the UK were screened for RANA viruses and RANA viruses were identified. A similar study made in the Chech republic on imported ornamental fish did not identify RANA viruses. Interestingly RANA viruses were identified in dead frogs from Denmark. According to the used risk assessment model it will not be possible to introduce EHNV to the EC, especially because no import of fish takes place from Australia. We had trouble confirming that redfin perch and rainbow trout can be infected by EHNV. Our studies questions if it is reasonable to list only EHNV and not the other RANA viruses and phylogenetic analyses illustrates that a new taxonomy might be needed.

Questions
***Birgit Oidtmann*:** What was the number of outbreaks in Australia?
Britt Bang Jensen: There were repeated outbreaks in redfin perch. 5-6 outbreaks occurred with very high mortality. In rainbow trout there was one outbreak showing mortality of about 0.5% in a farm located closely downstream of an infected redfin perch farm. Subsequent Australian infection experiments were made by IP.
***Richard Paley*:** In the pond with the infected Danish frogs, did you find RANA viruses in other animals?
***Britt Bang Jensen*:** There were only these amphibians in the pond. Two carps and snails were OK.
***Ana Afonso*:** The risk assessment model is based on expert opinions - and there are no notes on water contaminations. Was there any information of numbers regarding trade? And what were the origins and the species of imported ornamental fish examined in the Czech study?
***Thomas Vesely*:** Fish came from all continents and there were many different fish species.
***Britt Bang Jensen*:** Purpose to create the model figures and numbers will be included later – we looked at worst case scenario = 100% infected fish being imported.

Inter-laboratory Proficiency Test 2008

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Abstract: A comparative test of diagnostic procedures was provided by the Community Reference Laboratory for Fish Diseases (CRL) to 35 National Reference Laboratories (NRLs) in the middle of October 2008. The test was carried out according to ILAC-G13:2000 Guidelines for the

Requirements for the Competence of Providers of Proficiency Testing Schemes. The test contained five coded ampoules, with viral haemorrhagic septicaemia virus (VHSV), infectious haematopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV) or a mixture of VHSV and IPNV, respectively. The proficiency test was designed to primarily assess the ability of participating laboratories to identify the notifiable non-exotic viruses: VHSV and IHNV but also to assess their ability to differentiate other fish viruses, as IPNV, SVCV, perch rhabdovirus etc. The participants were also asked to titrate the viruses in order to assess the cell susceptibility for virus infection in the respective laboratories. In addition participants were encouraged to geno- and serotype isolates, and were asked to provide a full-length G-gene sequence of the rhabdovirus identified in the lowest numbered ampoule in the test. Participants were asked to reply latest December 12th 2008. Each laboratory has been given a code number to ensure discretion.

Outcome of Inter-laboratory Proficiency Test 2008

Identification of content: 22 participating laboratories correctly identified all viruses in all ampoules. 9 laboratories did not identify VHSV in ampoule IV. One laboratory did not identify IPNV in ampoule IV. Two laboratories found more isolates in an ampoule than were actually present. Two laboratories found SVCV in an ampoule when it was not present. 8 laboratories serotyped some isolates. 15 laboratories genotyped some isolates. 17 laboratories submitted sequences.

Methods applied: The general trend was that laboratories which applied more tests to identify samples, scored higher than those, which relied on fewer types of laboratory tests. 23 laboratories used ELISA for identification of viruses. 22 laboratories used IFAT for identification of viruses. 11 laboratories used neutralisation tests for identification of viruses. 29 laboratories used PCR for identification of viruses. 9 laboratories used other methods for identification of viruses. 29 laboratories used BF-2 cells. 33 laboratories used EPC cells. 12 laboratories used RTG-2 cells. 9 laboratories used FHM cells. 2 laboratories used CHSE-214 cells

Concluding remarks

In the ampoule containing a mixture of two viruses, only the IPNV and not the VHSV was identified by 9 laboratories. We encourage participants to be aware of the possibility of more viruses being present at the same time and that one can over grow the other on cell cultures, and thereby masking its presence.

The low performance in several laboratories of their RTG-2 cell lines for virus growth is worrying as is it described in Commission Decision 2001/183/EC that RTG-2 cells can be used instead of BF-2 cells. Based on these observations, we recommend that laboratories use BF-2 cells and not RTG-2 cells for replication/survey of/for VHSV.

PCR was the most frequently used method by participants identifying all viruses but also the method most frequently used by those participants obtaining the lowest scores. Another observation was that neutralisation is used by a relative high proportion of participating laboratories not obtaining highest score. Based on these findings we recommend participants to focus extra on evaluating how these two technologies are used for fish diagnostics.

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

Minutes

Report on the proficiency test 2008 is available at www.crl-fish.eu. Ampoule IV contained a mixture of VHSV and IPNV. 9 laboratories did not identify VHSV in ampoule IV. We encourage laboratories to be aware that more viruses can be present at the same time and that one virus can overgrow the other. Neutralisation tests were used by a relatively high proportion of laboratories

obtaining a low score. We recommend that laboratories focus extra on how these technologies are used for fish diagnostics.
Proficiency test 2009 will be sent out in the beginning of September 2009 – approximately one month earlier than normal. Identification of VHSV, IHNV and EHNV and differentiating from other viruses as IPNV, SVC and other Ranaviruses will be requested. Concerning ISA, KHV and EUS, we are still in the process of designing a proficiency test.

Questions:
Sven Bergmann: How to discriminate between EHNV and related RANA viruses?
Søren Kahns: We use sequencing, but it is difficulty since RANA viruses are very alike.
***Niels Jørgen Olesen*:** It is very much needed that a decision regarding taxonomi within this group of viruses is solved. For sure many laboratories will encounter difficulties in correct identification/ discrimination between EHNV and the other RANA viruses.
***Guiseppe Bovo*:** Do we have to take precautions about the distributions of the test?
***Niels Jørgen Olesen*:** The receiving lab will be responsible for biosecurity after arrival of the proficiency test.
***Guiseppe Bovo*:** I will be able to provide a Ranavirus antibody.
***Amedeo Manfrin*:** It would be nice to have recommendations in comments when receiving the answer on the proficiency test.
***Søren Kahns*:** The proficiency test is a way for the lab to control itself.
***Niels Jørgen Olesen*:** Each lab should decide before receiving how you expect to perform. We would not like to judge between labs.
***Hege Hellberg*:** The mollusc ringtest give recommendations.
***Niels Jørgen Olesen*:** If a lab underperforms for several years a mission to add support is often proposed.
***Søren Kahns*:** It can be difficult to identify the exact problem since we do not have all information.
***Olga Haenen*:** Cross contamination may take place when opening the ampoules with the saw, one after the other. We should realize this. To avoid contaminations, use a new saw, or disinfect the saw and cabinet totally between ampoules.
***Niels Jørgen Olesen*:** The ampoules have been changed in format due to old ampoules gave lower titres.
***Guiseppe Bovo*:** How do you control that all cell lines are what you think?
***Søren Kahns*:** This is a common problem for all cell lines. A lot of cell lines might not be what we think they are. Even if you buy form companies you can not be 100% sure. It can demand a large amount of work to find out the origin of the cell. The most important is that your cell lines are a sensitive tool towards identifying the virus. By participating in the proficiency tests you can test whether your cell lines can detect the pathogens at sensitivities as it should. If this is the case you do not have to worry so much.
***Sven Bergmann*:** As accredited labs we have to report if we work with something else than we state.

Technical report

Niels Jørgen Olesen briefly ran through the technical report 2008, which can be found at www.crl-fish.eu.

Workplan for 2009 and 2010

Niels Jørgen Olesen explained the objectives for 2009 and how these are being fulfilled. Workplan 2009 is available at www.crl-fish.eu/CRL_NRLs/Workplan.aspx. He then presented suggestions for workplan 2010:

- Update and include standard operating procedures on the CRL web page for the listed exotic and non-exotic diseases
- Expanding www.fishpathogens.eu with IHN, KHV and ISA
- Workshop and training courses in most updated diagnostic procedures for VHS, IHN, ISA and KHV
- Include ISAV and KHV in the annual proficiency test
- Assess the possibilities for organising proficiency test on diagnosis of EUS

***Birgit Claudia Oidtmann*:** Could you access specificity and sensitivity of diagnostic tests?
***Niels Jørgen Olesen*:** It's a heavy task to take up, but since it a very important point we will look more into this.

Annual meeting 2010

26-28 May 2010 was suggested.
Both Rumania and Århus (DK) were suggested as venues for next meeting.

***Martin Ruane Neil*:** You could maybe have the meeting in Denmark every second year and somewhere else every second year.

Pictures

Olga Haenen and Vlasta Jencic were excellent photographers during the workshop. For pictures from the workshop please have a look at www.crl-fish.eu/annual_meetings/photo_gallery.aspx.