Technical Report
2006

from the
Community Reference Laboratory
For Fish Disease

The National Veterinary Institute
Fish Disease Section,
Aarhus, Denmark

Content

Content of Annexes

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Introduction

The Danish Institute for Food and Veterinary Research (DFVF) is appointed as the Community Reference Laboratory for Fish Diseases (CRL), according to Commission Decision 2006/141/EC on financial aid from the Community for the operation of certain Community Reference Laboratories in the field of animal health and live animals 2005. The duties of the CRL are described in Council Directive 93/53/EEC of 24. June 1993 introducing minimum Community measures for the control of certain fish diseases (Annex C). A five year contract was signed in the Framework Partnership Agreement, No. SANCO/2005 FOOD SAFETY/010- Animal Health – Fish. These duties mainly concern fish diseases of list I: infectious salmon anaemia (ISA), and list II: viral haemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis (IHN). This report follows the format of the work programme adopted for the CRL for 2006, describing activities associated with each point and the status of ongoing projects. The list of functions and duties of the CRL follows this introduction.

During 2006, significant resources were used to establish and perform a training programme and capacity building in 5 selected National Reference Laboratories in EU. The programme consisted of 2 missions to each laboratory, organisation of a 1-week workshop, and the organisation of 3 proficiency tests for evaluation of progress. Significant progress was recorded and the very close monitoring was shown to be very effective. The final proof will be given by their performances in the comparative proficiency test which will be distributed in 2007 to all NRL´s in EU.

The permanent staff of the Fish Diseases Section in Aarhus, Denmark consist of approx. 20 academic and technical staff, primarily involved in research, diagnostics and consultancy with special focus on fish virology.

The 10th Annual Meeting of National Reference Laboratory for Fish Diseases (NRL) was held in our institute in Copenhagen, Denmark from the 22nd to the 24th of May 2006. There were 52 participants from 29 countries. It was thus the largest Annual meeting until know! Of the 25 EC Member States, 23 were represented. Cyprus and Portugal did not attend. In addition, Bulgaria, Romania, Faroe Islands, Iceland, Norway, and Switzerland were represented.
The first day was dedicated to present and recommend easily applicable criteria and standardised methods for molecular identification of notifiable fish pathogens while the second day focused on the update on important fish diseases in Europe and their control.

As the Commission asked us to relocate resources from producing a proficiency test to training 5 NRL´s in diagnostic techniques, no ringtest involving all laboratories were conducted in 2006.

Aarhus, Friday, 13 June 2008

Niels Jørgen Olesen and Nicole Nicolajsen

THE FUNCTIONS AND DUTIES FOR THE COMMUNITY REFERENCE LABORATORY FOR FISH DISEASES
Council Directive 93/53/EC – Annex C
Period: 1 January 2006 – 31 December 2006

1. LEGAL FUNCTIONS AND DUTIES

The functions and duties of the Reference Laboratory are described in the Council Directive 93/53/EEC, and are mainly concerned with fish diseases of list I and II in Council Directive 91/67/EEC.

The functions and duties of the Community reference laboratory for list I and II diseases shall be:

(a) To co-ordinate, in consultation with the Commission, the methods employed in the Member States for diagnosing the disease concerned, specifically by:

(b) Typing, storing and supplying strains of the pathogen of the relevant disease for serological tests and the preparation of antisera;

(c) Supplying standard sera and other reference reagents to the national reference laboratories in order to standardise the tests and reagents used in each Member State;

(d) Building up and retaining a collection of strains and isolates of the relevant pathogen;

(e) Organising periodic comparative tests of diagnostic procedures at Community level;

(f) Collecting and collating data and information on the methods of diagnosis used and the results of tests carried out in the Community;

(g) Characterising isolates of the pathogen of the relevant disease by the most up-to-date and appropriate methods to allow greater understanding of the epizootiology of the disease;

(h) Keeping abreast of developments in the surveillance, epizootiology and prevention of the relevant disease throughout the world;

(i) Retaining expertise on the relevant disease pathogen and other pertinent pathogens to enable rapid differential diagnosis;

(j) Acquiring a thorough knowledge of the preparation and use of the products of veterinary immunology used to eradicate and control the relevant disease;

1. To assist actively in the diagnosis of outbreaks of the relevant disease in Member States by receiving pathogen isolates for confirmatory diagnosis, characterisation and epizootic studies;

2. To facilitate the training or retraining of experts in laboratory diagnosis with a view to standardise diagnostic techniques throughout the Community;

3. To collaborate as regards methods of diagnosing list I diseases, with the competent laboratories in third countries where those diseases are prevalent.

Work programme for 2006

TECHNICAL REPORT

*1-2. Organise and prepare for the Annual Meeting for the National Reference Laboratories for Fish Diseases in 200*6

Organization of the 10th Annual Meeting

The 10th Annual Meeting of National Reference Laboratory for Fish Diseases (NRL) was held at DFVF in Copenhagen, Denmark from the 22nd to the 24th of May 2006. There were 52 participants from 29 countries. Of the 25 EC Member States, 23 were represented. Cyprus and Portugal did not attend. In addition, Bulgaria, Romania, Faroe Islands, Iceland, Norway, and Switzerland were represented.

The first day was dedicated to present and recommend easily applicable criteria and standardised methods for molecular identification of notifiable fish pathogens. We were aware that considerable research and validation is needed for most of the pathogens before standardisation is possible but the speakers were asked to present the most updated knowledge in their field. Considerable attention has been given to how to discriminate between putative non-virulent wild strains and highly pathogenic strains from aquaculture. The question has serious implication on legislation, surveillance and control strategies in the Community.
No final recommendations were given, but it appears that the views differs much according to the capacities of laboratories represented, as scientist involved in research on specific pathogens in general advocate for detailed discrimination while more basically equipped laboratories would prefer more simple and straight forward methods for discriminating between different genotypes of pathogens. Presentations were given on the following pathogens: VHSV (marine vs. freshwater), IHNV (high homology between strains), SVCV and all the SVCV-like isolates, ISA virus and the use of HPR typing for discrimination between non pathogenic wild type and farm isolates, *Gyrodactylus salaris* and how discrimination can be done based on genetic analysis.

The second day was primarily dedicated to the update on important fish diseases in Europe and their control, represented by individual speakers, as well as the annual update on the aquaculture production and the VHS/IHN situation in EU as collated by the CRL. This year Dr. Sandy Murray and Dr. Rob Raynard, FRS Aberdeen, agreed to provide a statistical analysis of the existing CRL data on Survey and Diagnosis for the past 10 years. In addition, Dr. Yngve Torgesen, EU Commission, presented trends of the aquaculture production in Europe based on public available databases. The data were compared to those collated via the annual questionnaire on surveillance and diagnosis organised by the CRL. Dr. Yngve Torgesen underlined the necessity of using official data for assessment of production capacity.
Presentations were given on the first VHS outbreak in Romania, and on the control and surveillance of VHS in Poland and Denmark, respectively.
The focus of the topic: *emerging diseases*, was this year on *Francisella sp.* infection in farmed Atlantic cod, the status of and diagnostic challenges of salmon alpha viruses and the occurrence of Herpes virus infections in carp in Europe. - All very comprehensive presentations.
Topics on technical issues related to diagnostic methods were also presented with reference to BKD and the viral diseases VHS, IHN, ISA, PD and IPN with emphasis on the effect of pooling organ material from more than one fish, comparative susceptibility studies, and assessment of various detection methods.

From the CRL, we presented the results of the proficiency test in 2005 and we informed that no test would be provided in 2006, as the CRL will be busy with

commitments to train a number of NRL´s in the autumn 2006. For the CRL work plan 2007 interest was expressed in enlarging the proficiency tests to comprise more pathogens (as ISA) and the possibility of dividing the test to cover different interests in different zones of EU (salmon producing, carp producing, Mediterranean production etc.).

Dr. Helle Frank Skall and Dr. Ellen Ariel took minutes from the meeting. Speakers have afterwards been given the chance to edit the minutes of their talk, thus hoping to keep misunderstandings to a minimum. This report refers in places to material, which was distributed during the meeting. The Annual Meeting was organised by a team consisting of Sanne Madsen, Nicole Nicolajsen, Helle Frank Skall, Ellen Ariel and Niels Jørgen Olesen with help from the remaining staff at the Section of Fish Diseases, DFVF, Århus and from Karen Damgaard Duun chief secretary, DFVF in Copenhagen.

The next Annual Meeting is planned for the 4th to the 7th of June 2007 at DFVF in Copenhagen.

A questionnaire for evaluation of the Annual meeting was provided at the end of the meeting. In general most participants were satisfied by the way the meeting was organised and we received a number of useful recommendations for improvements and many encouraging words on our work.

The final report, including programme and minutes of the meeting is enclosed as Annex 1

3. Collect data on the fish disease situation in EU.

Survey and diagnosis of VHS and IHN in 2005

Member States were asked to complete and return to the CRL a questionnaire on the Survey and Diagnosis of VHS and IHN in their home state during 2005. The questionnaire was composed of 3 parts: General data regarding production, epidemiological data and laboratory data.

It was noted that Spain isolated IHNV in 2005. Slovenia registered 4 farms as IHN infected. Bulgaria registered 1 farm as VHS infected. Only 6 of 697 farms in France were regarded as VHS or IHN infected.

A very significant number of samples are examined every year in the respective laboratories, and especially the new member states seem to have adopted regular laboratory diagnosis.

The number of isolations of other pathogens in 2005 was very similar to the numbers informed for 2004, and thus no obvious increase in known or emerging diseases is observed.

A significant number of other control programmes are conducted in each Member state, and most of these were registered in the new enlarged questionnaire for 2005.

The results were compiled and presented at the 10th Annual Meeting. In addition and based on a study of the results of theses questionnaires in the past 10 years Dr. Sandy Murray and Dr. Rob Raynard presented “An epidemiological update on the Survey and diagnosis in Europe based on 10 years annual registration”

A summary of the results for 2005 is presented in Annex 2

4. Identify and characterise selected virus isolates of ISA, VHS, IHN, and related viruses including the production of antisera against the isolates if necessary

Identify and characterise selected virus isolates

Again in 2006 a significant number of virus isolates were received for further characterisation and for including in our library of viruses:

Table 1: Material received at the CRL from laboratories in Member States and outside EU in 2006

Member States/ Countries outside EU		
Material	**Laboratories**	**Units**
Virus isolates	5	47 vials
Diagnostic material	12	83 samples
PCR material	1	45 samples
Other materiel	6	32 pieces

Further details are listed in Annex 3

Below is an account of the samples, isolates and reagents received for identification, characterization and update of the virus library and diagnostic procedures applied for the relevant cases:

England, CEFAS *(Peter Dixon and Barry Hill):* VHSV isolate, reference number J167, received for identification and characterisation. The G-gene of J167 was sequenced and aligned. The isolate was shown to belong to genotype Ia as other European freshwater VHSV isolates. The closest relatives were Danish VHSV isolates from 2005 and 2006 (DFVF-206184).

France, AFSSA *(Jeannette Castric):* IHNV and VHSV virus isolates were received for our virus library. Fish sera with antibodies against VHSV and IHNV for use in Plaque neutralisation tests were received as positive controls (DFVF-206261+206349).

Faeroe Islands, Food- veterinary and environmental agency *(Peter Østergaard):* Five samples were received for VHSV-, IHNV- and IPNV-examination (DFVF-206228). All were negative.

Italy, IZSVe *(Guiseppe Bovo):* Virological examination of virus 190/V06 infected SSN-1 cell culture, isolated from an organ pool of two ornamental fishes (redeye tetra *Moenkhausia sanctaefilomenae* and Harlequin rasbora *Trigonostigma heteromorpha*). The received virus isolate belong to the genus Birnaviridae. We could not confirm the isolate as IPNV (DFVF-206175).

Japan, Hokkaido University Hakodate *(Mamoru Yoshimizu) :* RTG-2 cell line and OMV isolated from rainbow trout, received (DFVF-206472).

Poland, NVRI *(Jerzy Antychowicz) :* Five VHSV isolates from rainbow trout from various regions of Poland were sequenced. All isolates clustered within Genogroup Ia (DFVF-206301).

Romania, Institute for Diagnosis and Animal Health *(Paul Dascalescu):* Four cell culture supernatants from samples of rainbow trout were received for virological examination and genetic characterisation. VHSV was isolated and the isolate was shown to belong to genotype Ia as other European freshwater VHSV isolates. The closest relatives were isolates from Switzerland, Austria, France and Denmark (DFVF-206051).

Russia, Kamchatka, ***(Aller Aqua):*** 12 samples from Chinook Salmon examined by immunohistochemistry (IHC) (DFVF-206298). No pathogens identified.

Slovakia, State Veterinary and Food Institute *(Miriam Revallová) :* Eight samples of fish from Slovakia were received for virological examination. It was not possible to isolate virus from the samples (DFVF-206371).

Spain, Laboratorio central de veterinaria (MAPA) (Pilar Fernández Somalo & Marta Vigo Martín)*:* IHN virus isolate from rainbow trout from Spain received for further identification and characterisation. The IHNV isolate belongs to Genogroup M, as other European isolates (DFVF-206240).

Switzerland, Centre for Fish and Wildlife Health Institute of Animal Pathology *(Thomas Whali):* 13 VHSV isolates from different locations in Switzerland were received for genetic characterization. All the isolates clustered within Genogroup Ia and were very similar to a number of Italian VHSV isolates (DFVF-206172 &DFVF-206103).

Sweden, SVA *(Suzanne Martelius-Walter):* Gills from eels in RNA-later and gills and kidney from eel in EMEM, no. SVA38-2006 were received for virological examination: *Herpesvirus anguillae* (HVA) was identified (first isolation in Sweden) (DFVF-206348).

Turkey, Bornova Veteriner Kontrol ve Arastirma Enstitusu *(Dr. Gulnur Kalayci & Dr. Serife Incoglu):* Five isolates received from rainbow trout from Turkey were identified as IPNV and to belong to serotype Sp.(DFVF-206161 to 206168). VHSV isolates from Turbot received for the library were unfortunately inactivated during transport.

Turkey, Ege Universitesi Su Uruntei *(Prof. Hasmet Cagirgan)* By virological examination of cell culture supernatants, from samples of rainbow trout from Turkey one IPN virus (TABA) was serotyped (Non Sp nor Ab) and a VHSV isolate (BOLU) was sequenced and shown to cluster Genogroup 1-e (the Black Sea VHS viruses) (DFVF-206239).

Czech Republic, National Reference Laboratory for Viral *(Tomas Vesely):* Monoclonal antibodies against SVCV-G 2E1 received (DFVF-206480).

USA Cornell University Ithaca *(Geof Groocock):* Virological examination of samples from round gobies (*Neogobius melanostomus*) received for virological examination and characterisation. The isolate was shown to belong to VHSV genotype IV as other North American VHSV isolates (DFVF-206151).

5. Optimization, standardisation and validation of RT-PCR for the diagnosis and identification of VHS

Validation of a RT-PCR assay for detection of viral haemorrhagic septicaemia virus (VHSV)

The aim of the validation of a RT-PCR assay for detecting the N-gene of VHSV is to standardise a technique witch enables to detect all strains of VHSV despite of genotypes and subtypes. In this work a panel of 12 different strains of VHS representing all known genotypes and subtypes were analysed for VHSV by RT-PCR using 3 different primer sets for comparison (Snow et al. 2004, Bergmann et al. unpublished, Madsen et al. unpublished).

The annealing temperatures were defined by gradient RT-PCR and the most suitable primers were used in the further validation.

The ability of the assay was analysed with regard to the detection limit, sensitivity, specificity, diagnostic sensitivity and diagnostic specificity.

A larger panel containing 50 VHS isolates have been selected and will be analysed in 2007.

Phylogenetic analysis and epidemiological tracing of viral haemorrhagic septicaemia virus (VHSV)

To study the genetic evolution of VHSV the entire G-gene from 74 isolates representing a broad geographic area has been characterised by Einer-Jensen et al. 2004. This work has been continued and today the phylogenetic analysis is based on more than 250 sequences of the entire G-gene of VHSV, which enables to characterise and compare VHS viruses and to assess epidemiological spreading of the disease.

The RT-PCR and sequencing is performed as described by Einer-Jensen et al. 2004.

According to computer analysis multiple sequence alignment of nucleotide sequences of the entire G-gene of VHS is performed with the

Clustal W program. The phylogenetic analysis is performed using the Phylip software and the Treeview software is used for construction of phylogenetic trees. Distance trees are generated by the neighbour-joining algoritm with Phylip. Furthermore neighbour-joining consence trees with defined outgroup is generated and to access the robustness of each node on the tree bootstrapping-resembling with 1000 replicates is performed. Bootstrap values exceeding 70% is considered to indicate significant relatedness.
Finally a more visible tree is performed by only including the closest related isolates and an isolate for each genotype and subtype.

6. Update and maintain a library of ISA, VHS and IHN virus isolates and entering this information into a database

Virus library

Several isolates for our library were received during 2006 (Annex 3). The library is continuously updated and maintained.

7. Maintain the electronic library for information material concerning prominent fish diseases

Electronic tutorials

Unfortunately, we did not have the capacity in 2006 to update the electronic library. The tutorials are placed in http://www.eu-crlfish.org/

8. Re-design and update the webpage for the CRL

CRL website

The CRL website is a notice board, where NRL´s and other interested parties can access information and previous reports concerning the activities coordinated by the CRL and relevant upcoming events in the Community. The address is http://www.eu-crlfish.org/ The homepage was redesigned in 2006. It was however decided not to make the homepage interactive by offering the possibility for direct communication and discussions on the page, as the capacity of the CRL for website maintenance is too restricted.

9. Supply standard antisera and other reference reagents to the National Reference Laboratories in Member States

Materials supplied by the CRL

On request, the CRL supplied material to other laboratories in Member States and third countries to aid in the diagnosis and characterisation of fish diseases. The number of laboratories receiving the specific material and the number of units supplied by the CRL are listed in table 2.

Further details are listed in Annex 4.

Table 2: The CRL supplied the following reagents in 2005

Material	Laboratories	Units
Cell cultures	9	38 flasks
Polyclonal antisera	5	16 vials
Monoclonal antisera	1	2 vials
Virus isolates	2	58 vials
Other material	3	9 vials/plates

10. Facilitate and provide training in laboratory diagnosis on the premises and on location, in particular for laboratories with special needs as determined after the previous Ring-tests

Introduction

For the past several years, the proficiency test provided by the Community Reference Laboratory for Fish Diseases (CRL) in EU to the 25+ National Reference Laboratories (NRL´s) has consistently showed that certain NRL´s are unable to identify notifiable viruses with a high degree of certainty. This is a concern for the validity of their disease certification and consequently elevates the risk of disease transfer with the trade of live fish. The EC Commission therefore approved the workplan for 2006 for the CRL to include special training in diagnostic techniques for 5 selected NRL´s. The Technical Assistance Information Exchange Office (TAIEX), DG Enlargement, European Commission agreed to provide funds for travel and subsistence for the participants during a specific workshop organised by the CRL in Aarhus, Denmark. TAIEX was responsible for the invitations, travel and accommodation and the CRL organised meals, lectures and laboratory exercises for the participants.

The workshop

After two CRL employes vivited all the 5 respective laboratories in a fact-finding-mission, the CRL proposed a training schedule together with the 5 NRL´s. The workshop on laboratory identification procedures for notifiable fish diseases took place at DFVF in Århus, Denmark during the 9th to the 13th of October 2006. NRL´s from all 5 countries were represented. The workshop provided 2 parallel programmes ("Cell culture" and "Identification") and the group of 10 participants (2 from each NRL) was divided into 2 teams, with each person attending the programme tailored to their area of responsibility or training needs. Details of the 2 programmes as well as names and contact details of participants are listed below.

The workshop consisted of a series of lectures and practical excersises adjusted to meet the specific needs of the participants. In house experts presented lectures and demonstrated laboratory techniques according to the most relevant viruses in the 5 countries. Participants were directly involved in performing the techniques. Experimentally infected fish displaying typical signs of VHS were provided for the participants to examine and diagnose using the following techniques: Cell culture techniques for isolation and quantification of virus, virus identification by means of enzyme linked immunosorbent assay (ELISA), serum neutralisation, immunoflourescence assay test (IFAT) and polymerase chain reaction (PCR). The techniques were trained in the context of solving the annual proficiency tests provided by the CRL to all NRL´s in EU according to Commission Decision 2001/183/EEC.

Follow up

Upon return to their own laboratory, the participants received a proficiency test to be solved within 3 weeks. For the second test, a CRL employee visited each of the 5 NRL´s for a follow up mission and assist in solving the test on location, in order to facilitate the implementation of the techniques in the respective laboratories. The 5 NRL´s will receive a further 2 proficiency tests before participating in the official 2007 proficiency test involving approximately 35 NRL´s in Europe and internationally

Preparation of Inter-laboratory Proficiency Test 2006

The Community Reference Laboratory provided two inter-laboratory proficiency tests on the quantification and identification of virus in 2006. The tests involved 5 NRL´s in the European Union, only, and were performed in connection with a special training course for these. Isolates of VHS-, IHN- and

SVCV virus as well as a blank were included in the tests. Five coded ampoules. The test was designed to assess the ability of participating laboratories to identify the notifiable viruses: VHSV and IHNV as well as SVCV. The first proficiency tests were sent at the end of October and the participants were asked to reply within 3 weeks of receiving the test.
The participated countries solved the second proficiency tests, when CRL visited them the second time.

Training and scientific collaboration
The following colleagues visited the institute during 2006 for scientific meetings, project collaboration or training

Dr. Pia Vennerström, EVIRA, Helsinki, Finland	16.05 - 19.05.2006
Dr. Alexandra Grilc, Veterinary Faculty of Ljubljana, Department of Virology, Slovenia	26.10 - 02.11.2006
Drs. Marek Matras, Mihael Reichert, Alicja Konzinaka, and Agnieszka Penela State Veterinary Institute in Pulawy, Fish Disease Department, Poland	14.08 - 24.08.2006
Drs. Siiri Tarrikas and Ülle Pau Veterinary and Food laboratory of Estonia, Ádám Dán and Tamás Juhász, Central Veterinary Institute, Budapest, Hungary, Drs. Liga Neretniece and Zita Muizniece, National Diagnostic Centre of Food and Veterinary ServiceRiga, Latvia, Vilija Jazukevicciute and Ingrida Jaceviciene National Veterinary Laboratory of the Republic of Lithuania, and Julia Habovstiaková and Miriam Revallová, State Veterinary and Food Institute. Slovakia	09.10 - 13.10.2006

11. Attending international meetings and conferences

Meetings and Conferences

Contact with colleagues from other laboratories is a channel for exchange of information in the field of fish disease, and an opportunity to keep abreast with new developments in the field. Of special interest are of course the activities relating to VHS, IHN and ISA. Scientists at the CRL participated in the following activities in 2006:

Presentations at international conferences and meetings

(* indicate presenting scientist, **bold** indicate scientist employed at the fish section at DFVF):

- ***Olesen N.J.*, Madsen S., Einar-Jensen K., Lorenzen N.*** **(2006)** VHSV: Not only VHSV? First International Conference of OIE Reference Laboratories and Collaborating Centres. Florianopolis, Brazil, 3-5 December
- ***Madsen S.*, Einer-Jensen K., Olesen N.J., Lorenzen N.*** **(2006)** Phylogenetic analysis and epidemiological tracing of viral haemorrhagic septicaemia virus (VHSV) SCOFDA (Sustainable Control of Fish Diseases in Aquaculture) workshop, KVL, Copenhagen, Denmark (6-7- November)
- ***Olesen N.J.*, Jensen B.B., Skall H.F.*** **(2006)** News on the BKD situation in Danish aquaculture SCOFDA (Sustainable Control of Fish Diseases in Aquaculture) workshop, KVL, Copenhagen, Denmark (6-7- November)
- ***Skall H.F.****, *Pedersen K, Lassen-Nielsen AM, Henriksen NH, Nielsen T &* ***Olesen NJ.*** **(2006):** Surveillance of fish diseases on eight Danish Marine farms during 2006 SCOFDA (Sustainable Control of Fish Diseases in Aquaculture) workshop, KVL, Copenhagen, Denmark (6-7- November)

- ***Madsen S.*, Olesen N.J.*** (**2006**) Results from sequencing of viruses at DFVF in 2006. EPIZONE 1st Progress meeting, DFVF, Copenhagen, November
- ***Madsen S.*.*** (**2006**) Results from the Sequencing of virus included in the Proficiency Test 2005. 10th Annual Meeting for National Reference Laboratories for Fish Diseases. DFVF, Copenhagen, 22-24th May
- ***Olesen N.J.*, Ariel E., Skall H.F & Nicolajsen N.*** (**2006**) Survey and diagnosis summary: 1) status and emergence of VHS and IHN in Europe 2) other fish disease monitoring programmes in EU 10th Annual Meeting for National Reference Laboratories for Fish Diseases. DFVF, Copenhagen, 22-24th May
- ***Skall H.F.**** **(2006)** Effect of pooling organ material on the diagnosis of BKD. 10th Annual Meeting for National Reference Laboratories for Fish Diseases. DFVF, Copenhagen, 22-24th May
- ***Skall H.F*.*** **(2006)** Organ tropism of *Renibacterium salmoninarum.*10th Annual Meeting for National Reference Laboratories for Fish Diseases. DFVF, Copenhagen, 22-24th May
- ***Skall H.F.*, Olesen NJ.*,*** *Ghiasi F & Bovo G.* **(2006)** Effect of pooling organ material on the isolation of VHS virus in naturally and experimentally infected fish.10th Annual Meeting for National Reference Laboratories for Fish Diseases. DFVF, Copenhagen, 22-24th May.
- ***Schyth B.D.*, Lorenzen, N.*** *& Pedersen, F.S.* **(2006)** Antiviral activity of small interfering RNAs: Specificity testing using heterologous virus reveals interferon-related effects overlooked by conventional mismatch controls, Keystone Symposium on RNAi and related pathways, Vancouver BC, Canada. January 2006
- ***Jensen B.B.*, Ersbøll, A.K., Korsholm, K., Olesen, N.J***. **(2006)** An epidemiological study of the occurence of Viral Haemorrhagic Septicemia in Denmark during 1982-2005. 11th International Symposium on Veterinary Epidemiology and Economics. Cairns, Australia, August
- ***Lorenzen N.**** **(2006)** The Successful DNA Vaccines In Fish: How Do They Work? 10th International Congress. International Society of Developmental and Comparative Immunology (ISDCI), July 1-6, 2006, Charleston, USA.
- ***J. Skou Rasmussen*, K. Einer-Jensen, E. Lorenzen and N. Lorenzen.*** **(2006)** Vaccination Of Rainbow Trout Against Viral Haemorrhagic Septicaemia Virus Using A Linear Dna Vaccine. 10th International Congress. International Society of Developmental and Comparative Immunology (ISDCI), July 1-6, 2006, Charleston, USA.
- ***K. Einer-Jensen*, E. Lorenzen, J. Skou Rasmussen and N. Lorenzen.*** **(2006)** The Importance Of N-Linked Glycans For Induction Of Protective Immunity To Vhs Virus In Rainbow Trout By The Viral Glycoprotein, When Delivered As A Dna Vaccine. 10th International Congress. International Society of Developmental and Comparative Immunology (ISDCI), July 1-6, 2006, Charleston, USA.
- ***K. Engell-Sørensen, N. Lorenzen.*** **(2006)** Improved Immunity of Aquacultured Animals. Status of the IP project IMAQUANIM. Presentation at the Workshop on Fish Farming Technologies for SMEs – Technological Challenges and Market Opportunity. Nov 23, 2006.

Scientific publications in peer-reviewed journals

- ***Schyth*, B.D., Lorenzen, N.*** *& Pedersen, F.S.* **(2006)** Antiviral activity of small interfering RNAs: Specificity testing using heterologous virus reveals interferon-related effects overlooked by conventional mismatch controls, Virology, 2006, 349: 134-141
- *Halli-Raja M, Vehmas TK, Rimaila-Pärnänen, Sainmaa S,* ***Skall HF, Olesen NJ*** *&Tapiovaara H* **(2006)**

Viral haemorrhagic septicaemia (VHS) outbreaks in Finnish rainbow trout farms. *Diseases of Aquatic Organisms* **72**, 201-211

- *Katrin Utke, Sven Bergmann,* ***Niels Lorenzen****, Bernd Köllner, Mitsuru Ototake and Uwe Fischer* **(2006)** Cell-mediated cytotoxicity in rainbow trout, Oncorhynchus mykiss, infected with viral haemorrhagic septicaemia virus. Fish & Shellfish Immunol. 22(3): 182-96. Epub May 10, 2006.
- *Acosta F, Collet B,* ***Lorenzen*** *N, Ellis AE.* **(2006)** Expression of the glycoprotein of viral haemorrhagic septicaemia virus (VHSV) on the surface of the fish cell line RTG-P1 induces type 1 interferon expression in neighbouring cells. Fish Shellfish Immunol. 21(3):272-8
- ***Einer-Jensen, K.****, Ahrens, P.,* ***Lorenzen, N.*** **(2006)** Genetic stability of the VHSV consensus sequence of G-gene in diagnostic samples from an acute outbreak *Bulletin of The European Association of Fish Pathologists* 26: 62-67

Other communications

- ***Schyth*, B.d., Lorenze****n, N. & Pedersen,* **(2006)** *F.S.* RNA interference in fish cells - small interfering RNAs targeting viral genes from a fish pathogenic virus. Marine station, Nanaimo, Vancouver Island, Canada .
- ***Lorenzen, N.*** **(2006)** Vaccination against VHS in rainbow trout: Experiments and perspectives related to practical fish farming. Abano, Italy
- ***Einer-Jensen, K****.* (2006) The importance of N-linked glycans for induction of protective immunity to VHS virus in rainbow trout by the viral glycoprotein, when delivered as a DNA vaccine. Seattle, WA, USA
- ***Ariel, E, Nicolajsen, N, HF.Skall, Olesen, N.J****.* **(2006)** Report of the 10th Annual Meeting of EU National Reference Laboratories for Fish Diseases, Copenhagen 22nd to the 24th of June.

12. Other work pertaining to the Functions and Duties of the CRL as described in Commission Decision 93/53/EEC

Other work at the CRL

Collaboration with colleagues from other laboratories is crucial for the CRL to keep abreast with new developments and is a channel for exchange of information in the field of fish disease. The group of scientists at the fish disease section of DFVF where the CRL is placed participated in the following scientific activities in 2006.

International scientific collaborative studies

- The group is partner in: EU concerted action contract number SSPE-CT-2003-502329. Development of a permanent network of experts on infectious diseases of aquaculture species for providing scientific advice on EU policy PANDA.
- In EU concerted Action: DIPNET funded under the Scientific Support to Policy initiative of the 6th Framework programme for research. Investigating disease interaction between wild and farmed fish,– the role of risk assessment and mathematical modelling. Contributions were especially given to Work Package 1: Review of disease interactions and pathogen exchange between wild and farmed fish in Europe.
- The group is project coordinator of EU project IMAQUANIM an Integrated Project Contract N°: 007103 « Fishing for new solutions towards improved health in the aquaculture sector ». The Project IMAQUANIM has brought together 17 universities and governmental research institutes, as well as five small and medium size enterprises (SMEs) working to develop technology to improve the disease immunity of Europe's major aquacultured species.
- The project “Innovative vaccines” is a collaborative study between 1) Istituto Zooprofilattico Sperimentale delle Venezie, Fish Pathology Department, Italy; 2) Clear Spring Foods (Research and Development), Idaho, USA; and 3) Danish Veterinary Institute, Denmark. Focus is on the improvement of DNA vaccines against two important disease causing fish

rhabdoviruses viral hemorrhagic septicemia virus (VHSV) and infectious hematopoietic necrosis virus (IHNV)

- The group is partner and project coordinator of EU project RANA : Risk assessment of new and emerging systemic iridoviral diseases for European fish and aquatic ecosystems. Proposal/Contract no.: 6459
- The group is partner and work package leader of EU project EPIZONE FP6-2004-Food-3-A WP 6.1: Surveillance & Epidemiology of emerging viral diseases in aquaculture.

Correspondence and Technical Consultation

Technical advice and consultation is considered by the CRL as one of its most important services. We attempt to answer requests as soon as possible and with the most up to date information available to us, or alternatively, refer to colleagues that are more involved in certain areas of fish disease research. Especially in the recent years focus on VHS increased significantly with the emergence of VHS in fresh water lakes in USA and Canada, with outbreaks in UK, Rumania, Turkey etc. The fast running e-mail correspondence is steadily increasing and the numbers of letters and contacts between the CRL and other laboratories have increased dramatically.

Research relating to fish disease taking place at DFVF

Fish immunology research activities of basic and applied character have been intensified with focus on host-pathogen interactions. The research activities of DFVF focus on VHSV infection in rainbow trout.

Improved Immunity of Aquacultured Animals (IMAQUANIM)
- an integrated research project in the EC FP6 programme-

IMAQUANIM includes 22 participants representing 9 European countries.
The project started on April 1, 2005 and has a duration of 5 years.
Project coordinator is the Danish Institute for Food and Veterinary Research.
Based on important disease models, this project concerns development of a technological knowledge platform for a future improved immunity to infectious pathogens in the major aquacultured species in Europe (Atlantic salmon, rainbow trout, sea bream, sea bass, carp, mussel and oyster). Focus will be put on use of vaccines, immuno-stimulants, immuno-diagnostic surveillance as well as markers for selection of the most immuno-competent individuals. Assays for qualitative and quantitative monitoring of key elements of the innate and adaptive immune system at genetic and functional levels will be established and used for determination of response profiles, which correlate with protective immunity. For all species, infection trials with various types of pathogens, including re-challenge of survivors, will be used to determine reference response profiles of naïve and primed animals

For finfish, vaccination trials with efficiently working commercial and experimental vaccines and corresponding control reagents will be used to identify critical response elements/profiles for vaccine efficacy. Variability both in terms of gene polymorphism and occurrence of isoforms among the immunological key elements and their regulation will be related to the functional response in *in vitro* assays as well as *in vivo* in terms of disease susceptibility of naïve and primed/vaccinated animals. The outcome of the project will be versatile gene array and immune response assays which can be implemented in development of efficient vaccines and immuno-stimulants for the finfish species and for genetic typing, monitoring of immuno-competence and immuno-diagnostic surveillance in both finfish and shellfish. In combination with the know-how established during the project these tools will

represent a strong platform for immunity-based reduction of losses caused by infectious diseases in future aquaculture.
Further info and updates are available at http://imaquanim.dfvf.dk/info/

Strategies to improve health and welfare in rainbow trout farming
A collaborative Danish research project including DFVF and 3 other research institutes as well as two commercial partners. The project is funded by the Department of Research and Development, Directorate for Food, Fisheries and Agri Business in the Ministry of Food, Agriculture and Fisheries of Denmark.
Project summary. This project will develop sustainable strategies that improve the health, welfare and quality of cultured rainbow trout by implementing three interrelated approaches: management, immuno-prophylactics, and selective breeding. The management approach will determine how increased water current at given rearing densities and water temperatures can reduce the stress of trout. The immuno-prophylactic approach will determine the relationship between immune response profiles and induction of protective immunity at different water temperatures and hereby establish efficient strategies for use of vaccination and feed stimulants in prevention of diseases. The selective breeding approach will determine how focus on physiological and immunological traits can improve the genetic basis for resistance of trout to stress and disease.
Further info: http://www.fishwelfare.dk/. Duration: 2005 – 2009.

Field-testing of a nucleic acid vaccine against VHS in rainbow trout.
A collaborative Danish research project including DFVF (project coordinator), the Danish Fish farmers Association and a small biotech company. The project is partly funded by the Department of Research and Development, Directorate for Food, Fisheries and Agri Business in the Ministry of Food, Agriculture and Fisheries of Denmark.
Project summary: A new and promising vaccine against VHS in rainbow trout has recently been developed by scientists at the Danish Institute for Food and Veterinary Research (DFVF) in collaboration with other research laboratories. The vaccine is a nucleic acid vaccine based on pure plasmid DNA produced in bacteria under stringent laboratory conditions. The purified vaccine is non-infectious and cannot replicate in the fish. Upon intramuscular injection, a dose of only one microgram vaccine can provide high, rapid and long lasting protection of rainbow trout fingerlings against VHS under experimental conditions. This is superior to any previous experimental VHS vaccines based on killed or attenuated virus or on recombinant protein. No negative side effects have been detected so far in the vaccinated animals, and compared to traditional types of vaccines, the new vaccine is considered to be of high safety. The biological principle behind the new vaccine is that some of the cells (muscle cells as well as other cell types) in the vaccinated animal will take up the injected nucleic acid construct. This construct encodes the gene for the viral surface glycoprotein G and will mediate expression of this protein in the host cells. Such cells will appear like virus-infected cells and will activate the immune defence like a virus infection, with the major difference that no infectious agent is involved. Although the vaccine is based on genes, the persistence of the vaccine in the fish is transient and since the vaccine is not transferred to the offspring, vaccinated fish are not considered as genetically modified according to the definitions given by EU. Clinical testing of similar types of vaccines against malaria, hepatitis and AIDS are presently being conducted in humans and a large field trial with a nucleic acid vaccine against another fish virus (IHNV) has recently been initiated with farmed Atlantic salmon in British Colombia, Canada.
Although intramuscular delivery of the vaccine is not the most convenient way

to vaccinate fish, it is expected that the VHS vaccine could be a valuable tool for reduction of losses caused by the virus under farming conditions. This includes not only prophylaxis against mortality in endemic zones but also as a part of eradication programmes where local periodical use of vaccinated fish would help to reduce multiplication of VHS virus in the fish populations and hereby facilitate elimination of the pathogen from the river systems.

Before initiating such practical use of the vaccine, it will be important to confirm that the vaccine also works under fish farming conditions, where the stress level and exposure to other pathogens is much higher than in a closed experimental facility. Permissions to perform a small scale field-testing was obtained from relevant public authorities including The Danish Medicines Agency, The Danish Forest and Nature Agency and The Danish Agency for Animal Experiments have been obtained.

A small scale field-testing has been conducted during two subsequent years. The fish were vaccinated during winther in enclosed setups at VHS-free farms. When VHS outbreaks ocurred at other farms during spring, subgroups of experimental fish were transferred to closed netcages in the diseased ponds. Mortality was monitored daily. A high cage-to-cage variability in mortality was observed when vaccinated and control fish were exposed to VHS in this manner. In some of the cages, there was a very significant protection against VHS among the vaccinated fish. In other cases, the cage setup increased incidence of other dicsease such as costiasis, and an equal high mortality among vaccinated and control fish was observed. The results are currently being processed and a final overview will be available in autumn 2007. a

Use of RNA interference for inhibition of expression of viral genes.

A PhD project supported by The Danish Research Councils and the Lundbeck Foundation

RNA interference by small interfering RNAs (siRNAs) is considered to be a highly specific method for knockdown of gene expression in eukaryotic cells via degradation of target mRNA. Efficiency of siRNAs designed to target viral mRNA has been tested in two models in vitro in carp EPC cells and in vivo in juvenile rainbow trout. It has been found that specific types of siRNAs and specific combinations of siRNA and transfection reagents are able to elicit a interferon response as measured by up-regulation of the antiviral interferon induced Mx molecule and induction of immune protection against virus. The usability of different types of controls has also been tested. Using siRNAs for inhibition of a fish pathogenic rhabdovirus, we observed that inclusion of a heterologous virus, as target control is essential for verification of the specificity of siRNA-induced interference with virus multiplication. Transfection with three different siRNAs specific to the viral glycoprotein gene of the target-virus efficiently inhibited viral multiplication in infected cell cultures, while two of three corresponding mismatched siRNAs did not have this effect. This suggested specific interference, but similar results were obtained when the same siRNAs were tested against the heterologous virus. Further analyses revealed that the siRNAs induced a non-target-specific anti-viral effect correlating with up-regulation of the Mx gene. Delivery to macrophage-like intraperitoneal cells has been accomplished by IP injecting chemically synthesized siRNAs complexed with the polycationic transfection reagent DOTAP. But this combination also appeared to be interferon inducing. The models will now be used for screening combinations of siRNAs, transfection reagents and delivery routes for their ability to specifically suppress viral replication without activating innate defence mechanisms like interferon.

Search for the origin of the vertebrate immune system.

This project is associated with the Danish Galathea 3 research expedition (www.galathea3.dk) and focus on basic fish immunology, potentially

complemented with screening for fish viruses in remote marine regions such as the Solomon Sea, the Antarctic zone, The Carribean and the Sargasso Sea. Fish represent the earliest class of vertebrates possessing the molecular key elements and functions of an adaptive immune system as known in higher vertebrates such as mammals, including man. By identification and characterisation of genes encoding key molecules of the fish immune system in a variety of both primitive and advanced fish species adapted to different life conditions, we hereby hope to create new knowledge of how the vertebrate immune system has developed in form and function and to relate this to the occurrence and evolution of disease-causing agents (pathogens).