Technical Report
2007

from the
Community Reference Laboratory
for Fish Diseases

Technical University of Denmark
National Veterinary Institute
Fish Disease Section,
Aarhus, Denmark

Content

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Introduction

The National Veterinary Institute, Technical University of Denmark (DTU-VET) is appointed as the Community Reference Laboratory for Fish Diseases (CRL), according to Commission Decision 2007/336/EC (OJEC L 128 of 16.05.2007) on financial aid from the Community for the operation of certain Community Reference Laboratories in the field of animal health and live animals 2007. 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 and confirmed by Specific Agreement No. 2007/2 to the Framework Partnership Agreement, No. SANCO/2005 FOOD SAFETY/005- Animal Health – Fish Diseases. The 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 2007, 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 2007, resources were used to organise a workshop on epidemiology and risk assessment as a consequence of the adoption of a new Council Directive 2006/88/EC to be finally implemented by August 2008; collating data on surveillance and diagnostics in the EU; organising and reporting the 11th Annual Meeting with many scientific contributions; organising an inter-laboratory proficiency test and report this; organising a workshop on serological methods; and validate molecular techniques for the identification of VHS virus.

The permanent staff of the Fish Disease 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.

In June 4-7 2007 the 11th annual meeting of the National Reference Laboratories for fish diseases was held back-to-back with a workshop in aquatic animal epidemiology and surveillance. A total of 65 participants from 34 countries attended over the four-day period. There were six sessions with a total of 34 presentations, 9 of which were given by invited speakers. Most Member States and several accession- and EFTA countries attended, either by sustaining from EU, TAIEX or on their own account. This was thus the Annual Meeting with the highest attendance ever.

The inter-laboratory proficiency test was taken up again this year and a report was submitted in March 2008. Most laboratories performed very well. This time we took the opportunity to ask participant to sequence the G-gene of the included rhabdoviruses in order to assess the uniformity of sequences of the same viruses sequenced in different laboratories. This was done to obtain a very important knowledge when assessing sequences for molecular tracing of the fish rhabdoviruses.

Aarhus, Thursday, 28 March 2008

Niels Jørgen Olesen , Søren Kahns and Nicole Nicolajsen

The functions and duties for the Community Reference Laboratory for Fish Diseases

According to Council Directive 93/53/EC – Annex C.
Period: 1 January 2007 – 31 December 2007

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. From 2008 the function and duties of the CRL described in Council Directive 2006/88/EC will be followed, as this Directive shall be fully implemented by August 2008.

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 2007

TECHNICAL REPORT

1-2. Organise and prepare for the Annual Meeting for the National Reference Laboratories for Fish Diseases in 2007

Organization of the 11th Annual Meeting

In June 4-7, 2007 the 11th annual meeting of the National Reference Laboratories for fish diseases was held back-to-back with a workshop in aquatic animal epidemiology and surveillance. A total of 65 participants from 34 countries attended over the four-day period. There were six sessions with a total of 34 presentations, 9 of which were given by invited speakers.

The scientific programme of the workshop was diverse and covered many topics of current interest. The first two sessions focused on the use of geographical information systems and molecular tracing and the concept of risk based surveillance in theory and practice, including a workshop where the participants discussed problems and solution to the implementation of the new council directive EC 2006/88, that prescribes surveillance based on risk assessment. This workshop was organised with the help of the International Society for Aquatic Animal Epidemiology, and the impression from the organisers is that we got around some interesting topics and there were many fruitful discussions, providing inputs for the European Commission. The workshop was terminated with a drinks reception sponsored by Bio-X, where all the participants had the opportunity to network and enjoy the nice Danish spring weather.

The annual meeting opened with the traditional session on update of fish diseases in Europe, where participants from the Member states presented new findings from their home countries. For the first time, UK had experienced an outbreak of VHS and presented the investigation done into this. The outbreak of VHS in the great lakes of the United States was also presented. This has caused severe mortalities and the identification of many new susceptible fish species.
This session was followed by a session on new or improved methods for diagnosis of the listed diseases, with both serological and molecular methods. Wednesday night the participants were invited to a banquet dinner in the old Sct. Nicolai church in the centre of Copenhagen.
The last day started with an update on scientific research carried out at some of the participating laboratories, where the results from PANDA and DIPNET-projects were presented and an update given on the development of a DNA-vaccine against VHS.
The annual meeting ended with the traditional update from the CRL, who gave a report from a year with focus on training of laboratories and the thoughts and considerations about implementing the new Directive and listed diseases in our work.

Minutes from the meeting were taken by Sanne Madsen, Helle Frank Skall and Britt Bang Jensen, 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 and 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 Britt Bang Jensen, Nicole Nicolajsen, Sanne Madsen, Helle Frank Skall and Niels Jørgen Olesen, with the help from the rest of the fish disease section at DTU-VET Aarhus. The meeting next year is planned for June 17-20, with focus on the exotic diseases listed in Council Directive 2006/88.

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, including the new listed diseases in the new Fish Directive: ISA, SVC and KHV

Survey and diagnosis in 2006

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 2006. The questionnaire was composed of 3 parts: General data regarding production, epidemiological data and laboratory data. The data regarding production during 1995 to 2005 was taken from "Fishery Statistical Collections Global Aquaculture Production (FIGIS)"

Special attention was given to new species in production and to fish species with large changes over the years. Generally, over the whole ten year period there has been an overall increase in production of 63%, but the total number has been steady the last four years.

A summary of the results for 2006 is presented in Annex 2a

Data on survey and diagnosis on fish diseases in Europe in 2006 were collected again this year. Compared to previous years some changes were made in the questionnaire. A reason for this is the implementation of the new Council Directive 2006/88/EF Annex IV part B where ISA, SVC and KHV were added to VHS and IHN on the list of non-exotic diseases. In addition, two new diseases, EHN and EUS, were added to the list of exotic diseases. These new diseases therefore got relatively more attention than in the previous S&D questionnaires.

For the zones approved free of IHN all Scandinavian countries + UK and Ireland are included while VHS appeared in UK in 2006 and is still present in Finland and Denmark. In the Northern Atlantic the only non-approved farms are situated at the Faeroe Islands. For the remaining countries, most of the "old" Member States have a number of farms approved in non-approved zones.

As in previous years, however, the de-facto spreading and significance of the non-exotic diseases cannot be retrieved from the S&D questionnaire.

A summary of the results for 2006 is presented in Annex 2b

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

Identify and characterise selected virus isolates

Again in 2007 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 2007

Member States/ Countries outside EU		
Material	**Laboratories**	**Units**
Virus isolates	7	26 vials
Diagnostic material	8	133 samples
PCR material	2	17 samples
Other material	3	38 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:

Austria, University of Veterinary Medicine *(Oskar Schachner)*: Virological examination of samples from rainbow trout ovarian fluid, protocol number PA07-675, fish number 4/07-4 (DTU-VET 207042). VHSV was identified.

Chile, Biovac S.A. *(Alejandra Aedo & Marcos Godoy)*: By RT-PCR examination of organ material in RNAlater from Atlantic salmon, all from case 25532, VHSV was not identified (DTU-VET 207265 and 207268).

Finland, Finnish Food Safety Authority, EVIRA *(Pia Vennerström)*: Sample Dn:o 29.11.2006/13 pa:8221 from perch from Finland is identified as perch rhabdovirus (DTU-VET 207010).

Serum samples from rainbow trout for identification of antibodies against VHSV (DTU-VET 2007-50-386).

Ireland, Marine Institute *(Lorraine McCarthy)*: Samples 3382 from farmed perch from Ireland identified as perch rhabdovirus (DTU-VET 207246).

Samples 3446 from farmed perch from Ireland identified as perch rhabdovirus (DTU-VET 2007-50-416).

Italy, IZSVe *(Guiseppe Bovo)*: 10 VHSV isolates received for sequencing (DTU-VET 207158).

Rabbit antisera against IPNV Sp and IPNV Vr-299 (DTU-VET 207049), received for validation.

Virusisolate from Italian catfish strain 24/0 (DTU-VET 207244) received (Rana virus).

Japan *(Mamozu Yoshimizu)*: Virusisolate no. VHSV T13/H15 - P3 (identical to TR-SW13G) and VHSV SW 136-P3 050507 (identical to TR-Bs13/15H) originating from Tyrkey (Nishizawa et al. 2006) (DTU-VET 207005). The isolates were verified as VHSV.

Oncorrhynchus Masou Virus (OMV) isolate 00-7812 (DTU-VET 207004), received for reference purpose.

Norway, National Veterinary Institute *(Brit Hjeltnes):* Organ material from 10 rainbow trout experiencing VHS outbreak (805/07, DTU-VET 2007-50-385). VHSV was isolated from all 10 fish. By sequencing the isolate was identified as belonging to genogroup III. By infection trial the isolate was shown to induce high mortality by immersion in rainbow trout.

Poland, National Veterinary Research Institute *(Marek Matres and Jerzy Antychowiez)*: Virusisolate from grayling (DTU-VET 207237). Unknown isolate, not VHSV, IHNV, IPNV or SVCV.

Virusisolate from brown trout (DTU-VET 207284). Unknown isolate, not VHSV, IHNV, IPNV or SVCV.

There is a link between the 2 farms from which the samples originate, as the brown trout were bought from the farm with the graylings.

Singapore, Agree-Food & Veterinary Authority of Singapore *(Lin Yueh Nuo)*: Irodovirus received for reference characterisation and reference purpose (DTU-VET 2007-50-344)

Sweden, Statens Veterinärmedicinska Anstalt *(Anders Hellström, Eva Säker and Suzanne Martelius-Walter)*: Sample U070524-0384 from farmed perch from Sweden was identified as perch rhabdovirus (DTU-VET 207192). Sample U070419-0156 was identified as IPN Ab and sample U070419-0158 and U070419-0261 was identified as IPN Sp (DTU-VET 207144).

The Netherlands, CIDC-Lelystad *(Olga L. M. Haenen)*: Phylogenetic analysis of VHSV samples CIDC-93489, CIDC-137609 and CIDC-133957 (DTU-VET 207046). All the isolates cluster within VHSV Genogroup Ia.

Phylogenetic analysis of VHSV sample CIDC-59670 (DTU-VET 207218).

Tyrkey, Bornova Veteriner Kontrol ve Arastirma *(Gulnur Kalayci and Serife Incoglu)*: Virological examination of cell culture supernatants, from samples of farmed rainbow trout and farmed sea bass from Turkey (DTU-VET 207111). VHSV was identified in cell culture supernatants from rainbow trout, and IPNV was identified in cell culture supernatants from rainbow trout and sea bass. Double infections were not observed.

UK-England, Cefas *(Amanda Bayley)*: Rana virusisolate GV6 and DFV received for reference purpose (DTU-VET 207256 and 207304) and samples for the KHV proficiency testing and KHV positive control H361 (DTU-VET 207292 and 207293) received from Keith Way.

5. Production of antisera against selected isolates if necessary

Production of antisera.

In 2007 no polyclonal or monoclonal antibodies were produced. Due to low stocks in our library, production and purification of SVCV was done in order to immunise rabbits for developing antiserum against SVC. Immunisation is in preparation in 2008.

6. Optimization and standardisation of RT-PCR and real-time PCR for the diagnosis and identification of VHS.

Optimization and standardisation of RT-PCR and real-time PCR for the diagnosis and identification of VHS.

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

The motive for validating a RT-PCR assay for detection of the N-gene of VHSV is to standardise a technique which enables to detect all strains of VHSV despite of genotypes and subtypes. The work included a panel of 12 different VHSV strains representing all published genotypes and subtypes. These were analysed by conventional 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 using a larger panel containing more than 70 VHSV isolates.
The ability of the assay was analysed regarding the detection limit, sensitivity and specificity and will be analysed regarding diagnostic sensitivity and diagnostic specificity.

Development of a Q-PCR assay for detection of viral haemorrhagic septicaemia virus (VHSV)

A diagnostic real-time RT-PCR assay was designed with the ability to detect and quantify all known genotypes and subtypes of VHS. The primers and probe were designed against an alignment consisting of more than 140 published sequences of the N-gene of VHSV.
The assay will be analysed regarding to its detection limit, sensitivity, specificity, diagnostic sensitivity and diagnostic specificity.

7. Update and maintain a library of Infectious salmon anaemia (ISA), Viral Haemorrhagic Septicaemia (VHS) and Infectious Haematopoietic Necrosis (IHN) virus isolates (including the sequences and GIS data of selected isolates) and entering this information into a *database.*

Virus library

Several isolates for our library were received during 2007 (Annex 3). The library is continuously updated and maintained.

8. Cooperate with existing projects concerning databases on viral genomes in order to obtain a functional and accessible system for molecular epidemiological tracing.

Cooperate with existing projects concerning databases on viral genomes in order to obtain a functional and accessible system for molecular epidemiological tracing.

FishPathogens.net is a resource to facilitate the tracking of pathogens of aquaculture within Europe for effective disease management in aquaculture. FishPathogens.net was established with the aim of collating available sequence information in order to make aligned data-sets relating to pathogens important for aquaculture readily available via a web-based interface.

The principal resource of FishPathogens.net is a database of nucleotide sequence data for aquatic pathogens.

Fishpathogens.net is a product of EUROPA (European Resource On the Pathogens of Aquaculture): a project funded by the European Union - Proposal Number QLRT-2001-02819.

The fishpathogens.net project was in development during 2003/4. Due to a technical problem and hosting/support issues, the site has not been online for over 12 months. In 2007, the original developer Tanya Gray was contracted by Mike Snow on behalf of FRS, Marine Laboratory, Aberdeen to update the web site, arrange web site hosting and complete related work with the aim to re-launch the web site. This work was taken over by the CRL at the end of the first contract and a new agreement between Tanya Gray will be signed for 2008.

The principal agreements were:

Fishpathogens.net scope will be extended to include isolate- and basic epidemiological information

Ownership of the database will accompany CRL wherever it goes.

Financial support for development has been provided by FRS and CRL. FRS, EPIZONE, EUROPA QLRT-2001-02819 and CRL are acknowledged on web site for its development.

Roles to support the application including report author, pathogen expert, CRL co-ordinator, technical administrator and support are agreed.

Second development phase will be based on agreement with CRL.

Tanya Gray continue in role as technical administrator and support.

For testing VHS will be used. CRL will be pathogen experts for VHS.

9. Maintain 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 partly redesigned in 2006. In 2007 it was however decided to design a new website being hosted at the DTU interface. This new website is planned to open just before the 12th Annual Meeting, in June 2008, where it will be presented for the NRL´s.

10. 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 2007

Material	Laboratories	Units
Cell cultures	7	28 flasks
Polyclonal antisera	7	17 vials
Monoclonal antisera	5	8 vials
Virus isolates	5	26 vials
Other material	2	102 vials

11. Prepare the Annual Inter-laboratory Proficiency Test year 2007 for the National Reference Laboratories.

Preparation of Inter-laboratory Proficiency Test 2007

A comparative test of diagnostic procedures was provided by the CRL to 35 NRLs in the end of September 2007.

The test contained five coded ampoules, with either viral haemorrhagic septicaemia virus (VHSV), infectious haematopoietic necrosis virus (IHNV) or spring viraemia of carp virus (SVCV). The ringtest was designed to primarily assess the ability of participating laboratories to identify the non-exotic viruses: VHSV, IHNV, and SVCV but also to assess their ability to differentiate other fish viruses, as infectious pancreatic necrosis virus (IPNV), perch rhabdovirus etc. In addition the participants were asked to titrate the viruses in order to assess the cell susceptibility for virus infection in the respective laboratories.

Participants were asked to reply within 8 weeks of receiving the test.

Due to an ongoing discussion on sequencing as a tool for differentiation between various genotypes of the non-exotic viruses, all laboratories were asked to provide full-length G-gene sequences of the submitted rhabdoviruses in the test. This exercise provides a good tool for assessing the quality of sequence data by assessing the homogeneity of the sequences. The results of the sequencing and genotyping were set 2 weeks after the first deadline.

Each laboratory has been given a code number to ensure discretion.

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

Outcome of Inter-laboratory Proficiency Test 2007

Identification of content

- 20 laboratories correctly identified all viruses in all ampoules.
- 11 laboratories did not isolate virus from ampoule II.
- 33 laboratories correctly identified the virus in ampoule I, IV and V
- 24 laboratories correctly identified the virus in ampoule II
- 34 laboratories correctly identified the virus in ampoule III
- 4 laboratories found double infection in one or two of the ampoules.
- 16 laboratories carried out sequencing and/or genotyping of the isolates.

Genotypning

Sixteen of the 35 laboratories provided sequence data but only ten participants attempted to genotype the viruses in the 5 ampoules. This may reflect a technical inability in the remaining laboratories, and if so, this is concerning in light of the ongoing discussion on genotyping as a basis for differentiation of notifiable viruses from others, and on including such typing in future legislation.

Ampoule I and ampoule II contained identical VHSV isolates: DK-F1 belonging to genotype I. All ten laboratories genotyped the virus correctly as type I.

Ampoule III contained the SVCV reference isolate 56/70 belonging to Genogroup I, subgroup Id. Twelve participants sequenced parts of the viral G-gene. Three participants correctly identified the virus as Id, two laboratories gave a virus reference without grouping the virus into Id. Only 6 laboratories thus attempted to type the virus despite the fact that this is to be done for correct

identification of SVCV.
Ampoule IV contained IHNV belonging to Genotype M. Fourteen participants obtained sequence data on the isolate. Five participants designated a name of the virus, provided a GenBank acc.no or submitted a phylogenetic tree, while only one laboratory directly indicated the virus as belonging to genotype M.

Ampoule V contained the marine VHSV isolate 4p101 belonging to genotype III. Fourteen participants submitted sequence data on this isolate. Of these, ten correctly designated the virus as a VHSV type III.

Especially, the SVCV and the IHNV isolates were designated according to alternative nomenclatures. For future studies it might be advisable to select one nomenclature that should be used in genotyping studies of SVCV and IHNV, e.g. as it is described by Stone et al. 2003 and Kurath et al. 2003, respectively).

In conclusion, the number of laboratories submitting an assessment of the genotypes included in the Proficiency test 2007 was not very impressive. Whether this low number was due to lack of technical skills or lack of resources allocated to the tests remain unknown. It is clear that the genotyping of VHSV is quite well established with only minor mistakes, while the genotyping of SVCV and IHNV were still not submitted the same way by all laboratories. Especially in the case of SVCV, where sequencing is demanded as a tool for definite identification of the notifiable disease SVC, the methods should be enforced. For IHNV, being a very homogenous virus, the typing is not crucial. For VHSV, the outcome of the discussions on splitting the VHSV genogroups in the legislation is very much dependent on the ability of all laboratories to be able to discriminate clearly between these.
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The full report is in Annex 5.

13. Facilitate and provide training in laboratory diagnosis.

Training and scientific collaboration
The following colleagues visited the institute during 2007 for scientific meetings, project collaboration or training.

Gabriele Vidgren, Finnish Food Safety Authority Evira, Finland. **My Khong Thi**, Centre for Fish- and Wildlife Disease, Switzerland. **Giuseppe Bovo**, Fish Pathology Department, OIE Reference Laboratory for Encephalopathy and Retinopathy, Istituto Zooprofilattico Sperimentale delle Venezie, Italy. **Erika Rampazzo**, Fish Pathology Department, OIE Reference Laboratory for Encephalopathy and Retinopathy, Istituto Zooprofilattico Sperimentale delle Venezie, Italy. **Peter Hostnik**, Veterinary Faculty, Institute for Microbiology and Parasitology – virology, Slovenia.	28th of November to 4th December 2007
Tanya Gray, Database developer, Symantix Ltd 91 Berkeley Road, Wroughton, Swindon, Wilts SN4 9BN. **Mike Snow FRS,** Marine Laboratory, Scotland.	7th to 8th of November 2007

14. Attending international meetings and conferences

International meetings and conferences attended.

Dr. N.J. Olesen participated in the EPIZONE FP6-2004-Food-3-A ½-year meeting in Lelystadt, NL, January 11-12 2007 and in the EPIZONE 1st Annual Meeting Lublin, Poland 29.05-01.06 2007 together with S. Madsen. He was invited speaker at the fish diseases conference "Frisk Fisk" in Tromsø, Norway, 22.01-25.01 2007 with the presentation: "Do VHS pose a risk for Norwegian aquaculture?". Dr. N.J. Olesen co-organised and was invited speaker at the 7th International Symposium on Viruses of Lower Vertebrates, Oslo, Norway, April 22 – 25, 2007, also attended by K. Einer-Jensen and B. Bang Jensen. He participated in the 13th International Conference on 'Diseases of Fish and Shellfish', 17th – 20th September 2007, Grado, Italy, also attended by H.F. Skall and Dr. E. Ariel. He was part of the Danish Galathea expedition, a round the world scientific cruise, from 27.03-15.04 2007 sailing from Sct Thomas to Boston on the project "The origin of the vertebrate immune system" conducted by Dr. N. Lorenzen; five scientists from our group participated in this project. Dr. Olesen was member of the EFSA Working Group on Fish Diseases vectors and participated in 3 meetings in Parma, Italy. He participated in the Workshop on wild animals health in countries around the Baltic Sea, 20th-22th of November 2007 in Tallinn, Estonia. Finally he is member of two working groups in the European Commission on risk based surveillance and diagnostic methods, respectively.

Dr H. F. Skall participated in the Co-ordination meeting of the Community reference laboratories (CRL) for feed and food control and animal health, Bruxelles. Following was on the program 1) Network of CRLs (regulation 882/2004) 2) State of play of designation of NRL's 3) Financial issues (regulation 1754/2006) 4) Ring trials 5) Training to TC's (Better training for safer food) 6) CRL scientific forum 13th of February 2007. Dr Skall participated in Introductory course on GIS and Spatial Analysis, KU-LIFE the 21-22 of August 2007.

Meetings and Conferences

Contact with colleagues from other laboratories is a channel for exchange of information in the field of fish diseases, 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 2007:

Presentations at international conferences and meetings

Dodge MJ, ***Olesen NJ****, Enzmann P-J & Stone DM* (2007) The occurrence of Viral Haemorrhagic Septicaemia (VHS) in England in 2006: A Molecular Epidemiological investigation. 13th International Conference on 'Diseases of Fish and Shellfish', 17th – 20th September 2007, Grado, Italy

Einer-Jensen K, Engell-Sørensen K, *Evensen Ø, Dalmo RA, Collet B, Fischer U, Scapigliati G, Alvarez MC, Mulero V, Tort L, Novoa B, Roch P, Venier P, Bovo G, Secombes C, Wiegertjes G, Vesely T, Buchmann K, Brudeseth B, Alonso S, Ingerslev E, Adams A, Slierendrecht W &* ***Lorenzen N*** (2007) Poster presentation at 7th International Symposium on Fish Immunology, 18-22 June 2007, Stirling UK. Improved Immunity Of Aquacultured Animals: The Imaquanim Project.

Einer-Jensen K, Engell-Sørensen K, *Evensen Ø, Dalmo RA, Collet B, Fischer U, Scapigliati G, Alvarez MC, Mulero V, Tort L, Novoa B, Roch P, Venier P, Bovo G, Secombes C, Wiegertjes G, Vesely T, Buchmann K, Brudeseth B, Alonso S, Ingerslev E, Adams A, Slierendrecht W &* ***Lorenzen N*** (2007) 13th International EAFP Conference on Diseases of Fish and Shellfish. Improved Immunity Of Aquacultured Animals: The Imaquanim Project.

Johansson T, ***Skall HF & Olesen NJ*** (2007) Molecular and phylogentic studies of perch rhabdoviruses (P-11). EAFP 13th International Conference on Diseases of Fish and Shellfish, Grado, Italy, September 2007

Madsen S, Jensen BB, Einar-Jensen K & Olesen NJ (2007) Phylogenetic analysis and epidemiological tracing of viral haemorrhagic septicaemia virus (VHSV). 13th International Conference on 'Diseases of Fish and Shellfish', 17th – 20th September 2007, Grado, Italy

Madsen S & Olesen NJ (2007) Status for Virus Characterization. EPIZONE 2nd Progress meeting, Pulawy, 30th May – 1stJune.

Madsen S & Olesen NJ (2007) Validation of a diagnostic RT-PCR assay for detection of viral haemorrhagic septicaemia virus (VHSV). 11th Annual Meeting for National Reference Laboratories for Fish Diseases, VET-DTU Copenhagen 4th –7th June.

Madsen S & Olesen NJ (2007) EPIZONE WP 6.1 Surveillance and Epidemiology of emerging viral diseases in aquaculture. Meeting for viologists at VET-DTU, Hjalet 3th – 4th October.

Haenen O & ***Olesen NJ*** (2007) EPIZONE questionnaire: global spread of koi herpesvirus by 2007. 13th International Conference on 'Diseases of Fish and Shellfish', 17th – 20th September 2007, Grado, Italy.

Lorenzen N *(*2007) DTU-VET, Oral presentation at local Research Seminar, Galathea 3 Expedition, Valparaiso, Chile, February 07, 2007. "Vaccination against VHS in rainbow trout: Experiments and perspectives related to fish farming".

Lorenzen N (2007) DTU-VET. Oral presentation at the CRL-meeting in Copenhagen June 07, 2007. "Vaccination against VHS in rainbow trout: Experiments and perspectives related to practical fish farming"

Lorenzen N, Lorenzen E, Rasmussen JS, Einer-Jensen K, Kjær TE*, Ingerslev E, Korsholm H & Henriksen NH* (2007). "DNA vaccination against VHS in rainbow trout: does it work under field conditions?" Nordic Symposium On Fish Immunology, Sterling, UK, June 19-22, 2007 Oral Presentation.

Lorenzen N (2007) "Use of recombinant single-chain antibodies in studies of anti viral immunity in fish". Oral presentation at Workshop on Antibody applications, linked with the Nordic Symposium on Fish Immunology, Sterling, UK, June 18, 2007

Lorenzen N, Rasmussen JS, Einer-Jensen K, Lorenzen E*, Christensen LH, Koch C & Lorenzen I* (2007) "Local protection against tetanus by intramuscular administration of plasmid DNA encoding a toxin-neutralizing single chain antibody". Poster presentation at "Antibodies 2007 - Europe" International conference, Vienna, Austria, November 7-8, 2007

Olesen NJ, Madsen S, Einer-Jensen K, Skall HF & Lorenzen N (2007) Classification of viral haemorrhagic septicaemia virus (VHSV) and how do we define the diseases VHS? The 7th International Symposium on Viruses of Lower Vertebrates, Oslo, Norway, April 22 – 25, 2007

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15. 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 DTU-VET, where the CRL is placed, participated in the following scientific activities in 2007.

Research relating to fish disease taking place at DTU-VET.

Fish immunology research activities of basic and applied character have been intensified with focus on host-pathogen interactions. The research activities of DTU-VET focus on VHSV infection in rainbow trout.: Regulation of gene expression including regulation by small microRNAs is studied in viral infected fish and during stimulated immune reaction. The use of synthethic small RNAs and immune stimulating components are investigated as means of prophylactic treatment against viral disease in trout. The work on RNA interference is done in collaboration with The siRNA Delivery Centre at the University of Aarhus.

The group was 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 (http://www.europanda.net/).

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 (http://ranavirus.net/).

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 (http://www.epizone-eu.net/default.aspx).

A 3-year national research project supported by the Danish Ministry of Food, Agriculture and Fisheries (FØTEK-4 programme), coordinated by the Section for Fish Diseases at DTU-VET and including collaboration with the Danish Fish Farmers Association (Danish Aquaculture), entitled "Field testing of a DNA vaccine for farmed fish" has been finalised. Despite variability and limitations in the small scale experimental setup, the overall results indicated that DNA vaccination against VHS in rainbow trout can induce protective immunity against the viral disease under field conditions. No negative side effects on the vaccinated fish were observed and no transfer of vaccine to the environment was detected. The next step towards implementation of DNA vaccines in European Aquaculture should include a full scale clinical testing. This will imply that the vaccinated fish must be allowed to reach the food chain and since no DNA vaccines have been licensed for husbandry animals in Europe yet, this step will require initial acceptance by the food safety authorities. Although a sceptical public opinion against use of gene-modified elements in food production exists in some countries, this should be achievable since all experiments with DNA vaccines in both animals and humans have so far supported the view that the risk of negative side effects is very small, also when compared to those observed for traditional vaccines.

The group is co-ordinating a 5 year EC-supported FP6 integrated project including 22 participants in nine European countries. The project is entitled "Improved immunity of aquacultured animals" (IMAQUANIM) and has successfully passed the midterm evaluation. The work includes both basic fish immunology research and applied research and technical development for establishment of a platform of knowledge and tools for better disease prophylaxis in cultured fish and shellfish. A report summary of the research activities and results including publications is available at the project website http://imaquanim.dfvf.dk/info/.

"Use of RNA interference for inhibition of expression of viral genes" is a project supported by The Danish Research Councils and the Lundbeck Foundation: The recent discovery, that small regulatory RNAs act in diverse roles of the eukaryotic intracellular life such as fine-tuners of endogenous gene expression, inhibitors of viral replication and as suppressors of transposon activity, is now widely recognized as a major breakthrough in our understanding of cellular biology. The pathways used by these small RNAs all seem to build upon a few central mechanisms in which small double stranded RNAs are generated from various precursor RNAs of exogenous or endogenous origin and used for inhibiting expression of genes with sequence complementarity to the small RNA. In particular, one class of small gene regulatory RNAs, known as small interfering RNAs (siRNAs), have the potential for highly specific gene silencing. These are 21 nucleotide perfectly complementary double stranded RNAs with 3´ end overhangs. 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 are 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. Another class of naturally found small regulatory RNAs the microRNAs are studied with respect to their expression and function in fish cells or fish infected with virus or stimulated to generate immune responses. There are two aims of these studies: 1) investigation of the host-pathogen interaction and diagnosis of the stage of disease and 2) development of treatment strategies based on the natural defence of the host.

“Search for the origin of the vertebrate immune system”.This project was 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 Caribbean 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).

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, Bulgaria, Norway 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 significantly in the recent years.