European Union Reference Laboratory for Fish Diseases
National Veterinary Institute, Technical University of Denmark, Aarhus

DTU

EURL-Fish work done in 2013

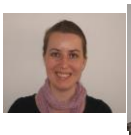
Niels Jørgen Olesen, *Niccolò Vendramin, Susie Sommer Mikkelsen, Anemone Ojala, Morten S. Bruun and Torsten Boutrup*

EURL-Fish in 2013

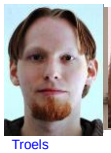
The year of transition from Aarhus to Copenhagen.

The New Fish Team








Morten S. Bruun

Niccoló Vendramin

Susie Sommer Mikkelsen

Torsten S. Boutrup

??

Betina

Christina

Troels

Anemone

EURL-Fish work program 2013

5 main objectives

- **1. Coordination and training**
- **2. Proficiency test**
- **3. Reagents and products**
- **4. Scientific advice and activities**
- **5. Missions**

20 subgoals

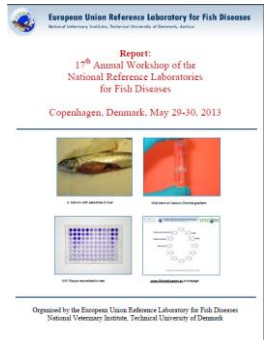
EURL-Fish work program 2013

5

1-1	**Annual workshop**	Organise and prepare for the 17th Annual Workshop for the National Reference Laboratories for Fish Diseases (NRLs) in 2013
1-2	**Annual workshop report**	Produce a report from the Annual Workshop 2013.
1-3	**Survey& diagnosis**	Collect and report data on the fish disease situation in EU, including all the listed non-exotic fish diseases given in Council Directive 2006/88/EC Annex IV Part 2.
1-4	**Training**	Facilitate and provide training in laboratory diagnosis. The yearly training courses in methods used for diagnosis of fish diseases will be offered at the EURL laboratory facilities. The courses will primarily be for training of staff from NRLs and the content will depend on request from participants.
2-1	**Proficiency test**	Prepare the Annual Inter-laboratory Proficiency Test year 2013 for the NRLs. The test will include VHSV, IHNV, EHNV, ISAV, KHV and Aphanomyces Invadans.
2-2	**PT report**	Collate and analyse information gained from the Inter-laboratory Proficiency Test
3-1	**Reagents**	Supply reference reagents to the NRLs in Member States.
3-2	**Antisera**	Production of antisera against selected isolates when necessary
3-3	**Pathogen library**	Update and maintain a library of isolates of Infectious salmon anaemia virus (ISAV), Viral Haemorrhagic Septicaemia virus (VHSV) and Infectious Haematopoietic Necrosis virus (IHNV), Koi Herpes virus (KHV) and enzootic haematopoietic necrosis virus (EHNV) and Aphanomyces Invadans.
3-4	**Tissue library**	Maintain a library of tissue material from fish infected with listed pathogens
4-1	**Webpage**	Update the webpage for the EURL, www.eurl-fish.eu
4-2	**Diagnostic manuals**	Update the diagnostic manuals for VHS, IHN, ISA, KHV disease, EHN and EUS on the EURL web page.
4-3	**Fishreflabnet**	Establish an interactive network with the NRLs, Fishreflabnet , in order to promote a more proactive data sharing and communication with and between reference laboratories in member states.
4-4	**Pathogen characterization**	Identify and characterise selected isolates of listed viruses (pathogenicity testing in vivo and in-vitro, serological and genetic characterisation).
4-5	**www.fishpathogens.eu**	Update and expand www.fishpathogens.eu with more pathogens.
4-6	**Molecular epidemiology**	Perform molecular epidemiology analysis to improve knowledge on diseases spreading mechanisms of viral pathogens.
4-7	**Real-time PCR**	Assessment and standardisation of real-time PCR tests for the diagnosis, identification and typing of the listed non-exotic fish diseases.
4-8	**Emerging diseases**	In collaboration with specialised experts WW to review selected emerging fish diseases in Europe and assess their potential listing as exotic or non-exotic diseases (e.g. using discontools and similar tools)
5-1	**Missions**	Organizing missions to relevant laboratories. Missions will focus on NRLs where on-site communication would be beneficial.
5-2	**International meetings**	Attending missions, international meetings and conferences in order to be updated on emerging and listed exotic and non-exotic fish diseases.

1-1,1-2 Organise and prepare for the 17th Annual Meeting for the NRLs and produce a report from the Annual Meeting

6

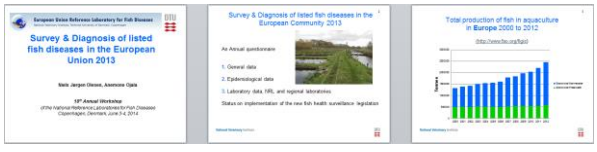


National Veterinary Institute

DTU

1-3 Collect and report data on the fish diseases situation in EU

7



National Veterinary Institute

DTU

1-4 Facilitate and provide training in laboratory diagnosis:

8

EURL training courses 2013

1) 5-days course in **"Sampling and diagnostic procedures for surveillance of listed fish diseases"** was primarily based on practical work (hands on) in combination with theoretical presentations.

A 4-day course in **"Molecular techniques and bio-informatics"** was devoted to lectures on relevant topics as well as theoretical exercises

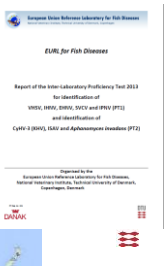
Aarhus, 21/1-31/1 2013 organised by the EURL for Fish Diseases

National Veterinary Institute

9

2-1, 2-2 Prepare and report the Annual Inter-laboratory Proficiency Test.

PT1-2013 for identification of:
VHSV, IHNV, EHNV, SVCV and IPNV
and PT2 identification of:
CyHV-3 (KHV), and ISAV and *Aphanomyces invadans*



10

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

Materials supplied by the EURL

On request, the EURL supplies material to other laboratories in Member States and Third Countries to aid in the diagnosis and characterisation of fish diseases.

11

3-2 Production of antisera against selected isolates when necessary

In 2013 antisera against Nodavirus was produced in rabbits. In addition new stocks of supernatants from Hybridoma cells producing monoclonal antibodies against VHSV (IP5B11) were produced.

12

3-3 Update and maintain a library of isolates of ISAV, VHSV and IHNV, KHV and EHNV

- Isolates of the listed viruses VHSV, IHNV and KHV were received and stored in our library during 2013.
- The EURL received 6 samples for PCR testing for KHV and 1 sample for IPNV and 2 samples were tested for Sarcocystidae infection. PRV, positive control material for PCR was received and our library have continuously been updated and maintained. .

13

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

For use as tissue library of positive naturally infected tissue from VHSV, IHNV and IPNV infected fish, organ pieces has been collected, stored and maintained, as well as organ material from negative controls.

Tissue material from rainbow trout infected with IPNV, salmonid alphavirus (SAV), *Oncorrhynchus masou* virus (OMV) and *Aphanomyces invadans* (EUS) have been stored as well.

14

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

The EURL website is a notice board, where NRLs and other interested parties can access relevant information and previous reports concerning the activities coordinated by the EURL and relevant upcoming events in the Community.
A new design was created in 2013
The **e-mail group VET-EURL** with approximately 100 colleagues subscribing is updated. The site is used for newsletters, scientific updates and announcements from the EURL Fish.

15

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

- The diagnostic manuals for VHS, IHN, ISA, KHV, EHN disease and EUS have all been prepared and are available from the EURL web page.
- The diagnostic manuals for VHS and IHN are updated and modifications of Commission Decision 2001/183/EC made.
- The diagnostic manual for ISA was prepared based on Commission Decision 2003/446/EC.
- The KHV and ISA manuals have been updated and changed significantly
- In all five manuals with the latest information on test developments as analytical sensitivity and specificity are included.

16

4-3 Fishreflabnet:

-
- A new e-mail group was created in 2012: VET-EURL with approximately 100 colleagues subscribing. Official communication and updates of interest to the scientific community are delivered periodically.
- Furthermore this tool is used for newsletters, scientific updates and announcements from the EURL Fish like announcements and invitations for the Annual Workshop or publication of content in the ampoules from the proficiency test or on the final Inter-laboratory Proficiency test report.

4-4 Pathogen characterization: Identify and characterise selected isolates of listed viruses

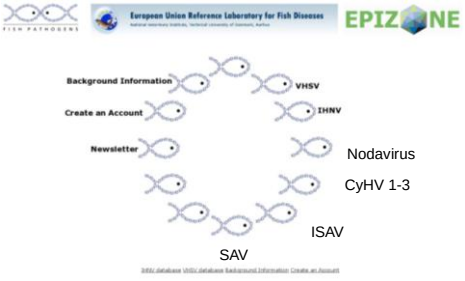
- **VHSV:** The full length G-genes of a large number of Danish VHSV isolates (>170) were sequenced by FLI, Germany (Dr Heike Schuetze) and aligned for molecular tracing of passed VHS outbreaks in Denmark. In total > 200 VHSV isolates were sequenced
- **KHV:** Diseases of Aquatic Organisms: *Marc Y. Engelsma, Keith Way, Melanie J. Dodge, Michal Voorbergen-Laarman, Valentina Panzarin, Miriam Abbadi, Mansour El-Matbouli, Helle Frank Skall, Søren Kahns, David M. Stone* (**2013**) Detection of novel strains of cyprinid herpesvirus closely related to koi herpesvirus.

4-4 Identify and characterise selected isolates of listed viruses

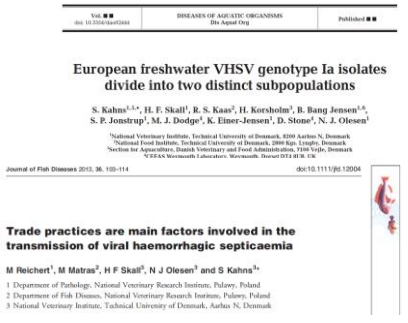
- Again in 2013 a significant number of virus isolates were received for further characterisation at the EURL and for storing in our virus library

Member States and countries outside EU		
Material received	**Laboratories**	**Units**
Diagnostic material for virology	**3**	**9 samples**
Diagnostic material for PCR	**1**	**6 samples**
Diagnostic material for bacteriology	**1**	**6 samples**
PCR control material	**1**	**1 sample**
Serum	**1**	**119 samples**
Other material	**1**	**2 samples**

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



4-6 Perform molecular epidemiology analysis to improve knowledge on diseases spreading mechanisms of viral pathogens.



European freshwater VHSV genotype Ia isolates divide into two distinct subpopulations

S. Kahns[1,3,*], H. F. Skall[1], R. S. Kaas[2], H. Korsholm[3], B. Bang Jensen[1,3], S. P. Jonstrup[1], M. J. Dodge[4], K. Einer-Jensen[1], D. Stone[4], N. J. Olesen[1]

Journal of Fish Diseases 2013, 36, 103–114 doi:10.1111/jfd.12004

Trade practices are main factors involved in the transmission of viral haemorrhagic septicaemia

M Reichert[1], M Matras[2], H F Skall[3], N J Olesen[3] and S Kahns[3,*]

21

4-7 Assessment and standardisation of real-time PCR tests.

Two new real-time PCR's has been has been assessed for the detection of the emerging diseases PMCV (Piscine myocarditis virus) and PRV (Piscine Reovirus).

4-8 Review selected emerging fish diseases in Europe and assess their potential listing as exotic or non-exotic diseases

New putative emerging disease in Sweden
Listing of EUS?
Pancreas disease and sleeping disease caused by the salmonid alphaviruses, SAV-1/ SAV-3 and SAV-2, respectively

22

5-1 Organizing missions to relevant laboratories.

In 2013 no missions

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

23

19th Annual Meeting 2015

- When?
- Where?
- Additional scientific workshop ?