Report of the Workshop in Aquatic Animal Epidemiology and Surveillance and 11th Annual Meeting of EU National Reference Laboratories for Fish Diseases

Copenhagen, Denmark
June 4-7, 2007

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

Contents

Introduction and short summary

In June 4th-7th 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 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 describes surveillance based on risk assessment. This workshop was organised with the help of 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 once again 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. We also heard about the outbreak of VHS in the great lakes of the United States, which 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 labs, 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 Nicolaisen, Sanne Madsen, Helle Frank Skall and Niels Jørgen Olesen, with the help from the rest of the fish disease section at VET-DTU Aarhus.

The meeting next year is tentatively planned for June 16th-18th, with focus on the exotic diseases listed in Council Directive 2006/88, but more details will follow.

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

Århus, 13 August 2007

Niels Jørgen Olesen and Britt Bang Jensen

Programme

Workshop in Aquatic Animal Epidemiology and Surveillance and 11th Annual Meeting of the National Reference Laboratories for Fish Diseases

4-7 June 2007

National Veterinary Institute, Technical University of Denmark, Copenhagen, Denmark

Programme

Monday 4 June – Workshop in Aquatic Animal Epidemiology and Surveillance

REGISTRATION AND WELCOME ADDRESS

13.00 - 14.00	Workshop Registration *Introduction to Workshop* - Niels Jørgen Olesen and Britt Bang Jensen
SESSION I:	GIS AND MOLECULAR TRACING *Chair: Olga Haenen*
14.00 - 14.45	The use of GIS for spatial analysis of spread of *Gyrodactylus salaris* - *Peder Jansen*
14.45 - 15.15	EPIZONE WP6.1: Surveillance and Epidemiology of emerging viral diseases in aquaculture – intentions and purpose - *Niels Jørgen Olesen*
15.15 - 15.45	COFFEE
15.45 - 16.15	A proposal of a GIS data model to support the aquatic animal disease surveillance **-** *Nicola Ferré*
16.15 – 17.05	The first occurence of Viral Haemorrhagic Septicaemia (VHS) in England – *Kevin Denham* The 2006 VHS Outbreak in England – Epidemiological Investigation into the origin of infection - *Birgit Oidtman*

Tuesday 5 June – Workshop in Aquatic Animal Epidemiology and Surveillance Continued

SESSION IIA:	RISK BASED SURVEILLANCE *Chair: Brit Hjeltnes*
9.00 - 9.30	The New Aquatic Animal Health Directive on Surveillance and its Implementation - *Sigrid Cabot*
9.30 – 10.40	What is risk-based surveillance and how does it apply to the control of fish health? - *Birgit Oidtman*
10.40 - 11.00	COFFEE

11.00 - 11.45	Diagnostic testing /sensitivity/specificity - *Marios Georgiadis*
11.45 - 12.15	The new OIE guidelines for aquatic animal health surveillance - *Barry Hill*
12.15 – 12.45	Implementation of risk-based sampling methods and sample size in the surveillance to document freedom from IBR in the Danish cattle population - *Håkan Vigre*
12.45 - 13.45	LUNCH
13.45 - 14.30	Demonstration on the UK live fish database - *Caroline Crane*

SESSION IIB:	EXAMPLES OF PRACTICAL APPLICATIONS OF RISK BASED SURVEILLANCE *Chair: Britt Bang Jensen*
14.30-15.00	Introduction to group discussions on how to apply risk based surveillance for specific regions and diseases
15.00-16.30	Group discussions on how to apply risk based surveillance for specific regions and diseases ➢ Eastern Europe ➢ Northern Europe ➢ South Europe ➢ Improvement of passive surveillance
16.30-17.30	Presentations and discussion of the outcome of the group-work

18.00	Drinks Reception and Registration

Wednesday 6 June – Annual Meeting of the National Reference Laboratories

WELCOME ADDRESS AND ANNOUNCEMENTS

9.00 - 9.30 *Dr. Niels Jørgen Olesen (Community Reference Laboratory)*

SESSION III:	UPDATE ON IMPORTANT FISH DISEASES IN EUROPE AND THEIR CONTROL *Chair: Rob Raynard*
9.30 - 10.00	Trends in Aquaculture production in Europe - *Britt B. Jensen*
10.00 - 10.30	Overview of disease situation in Europe - *Niels Jørgen Olesen*
10.30 - 10.45	VHS outbreaks in Switzerland in 2006 - *Thomas Wahli*
10.45 - 11.00	Experiences with VHSV diagnosis in Austria 2006 - *Oscar Schachner*
11.00 - 11.15	Update on the current status of VHS and IHN in Spain - *Marta Vigo*
11.15 - 11.45	COFFEE
11.45 - 12.00	Fish health trends and development in norwegian aquaculture 2006 - *Hege Hellberg*
12.00 - 12.15	KHV in Germany - *Sven Bergmann*
12.15 - 12.30	Occurrence of a new subtype of North American viral haemorrhagic septicaemia virus (VHSV) in the Great Lakes - *Helle Frank Skall*
12.30 - 12.45	Disease problems in the mediteranian - *Guiseppe Bovo*
12.45 - 14.00	LUNCH

SESSION IV:	**TECHNICAL ISSUES RELATED TO DIAGNOSIS** *Chair: Sven Bergmann*
14.00 - 14.20	Validation of serological methods used for diagnosis of VHS and IHN - *Jeanette Castric*
14.20 - 14.40	Validation of a RT-PCR assay for identification of viral haemorrhagic septicaemia virus (VHSV) - *Sanne Madsen*
14.40 - 15.00	Classification of viral haemorrhagic septicaemia virus (VHSV) and how do we define the disease VHS? - *Niels Jørgen Olesen*
15.00 - 15.30	The use of VHS-IHN samples for screening of other fish pathogens - *Hege Hellberg*
15.30 - 16.00	COFFEE
16.00 - 16.20	Sampling and diagnostic guidelines for ISA - *Brit Hjeltnes*
16.20 - 16.40	EPIZONE: Results of global Koi Herpes Virus questionnaire - *Olga Haenen*
16.40 - 17.00	Koi Herpesvirus – sampling, diagnosis and results of the 2006 PCR ring trial. - *Richard Paley*
17.00 - 17.20	*Confirmation of Spring viraemia of carp virus* - David Stone
17.20 - 17.40	Detection of *Campylobacter* in poultry - aspects of diagnostic PCR - *Marianne Lund*

19.00	BANQUET DINNER

Thursday 7 June– Annual Meeting of the National Reference Laboratories
Continued

SESSION V	**SCIENTIFIC RESEARCH UPDATE** **Chair: Giuseppe Bovo**
9.00 - 9.30	Vertical transmission of ISA – *Knut Falk*
9.30 - 9.50	PANDA: Diagnostic methods of disease hazards to European aquaculture - *Olga Haenen*
9.50 - 10.15	Highlights from the DIPNET Project - Disease interactions and pathogen exchange between farmed and wild aquatic animal populations – A European Network - *Rob Raynard*
10.15 - 10.45	Vaccination against VHS in rainbow trout: Experimental testing and perspectives related to practical fish farming - *Niels Lorenzen*
10.45 - 11.15	**COFFEE**

SESSION VI:	**UPDATE FROM THE CRL** **Chair: N.J. Olesen**
11.15 - 11.40	Training for laboratories with special needs in 2006. Protocol for management of underperformance/lack of collaboration of National Reference Laboratories (NRLs) in comparative testing and lack of collaboration with CRL activities - *Helle Frank Skall*
11.40 - 12.00	Report from Year 2006 - *Niels Jørgen Olesen*
12.00 - 12.20	Workplan for 2007 and 2008 - *Niels Jørgen Olesen*
12.20 - 12.40	CRL in the future – 5 listed diseases and ringtests - *Niels Jørgen Olesen*
12.40 - 13.00	Next meeting and end of 11th Annual Meeting - *Niels Jørgen Olesen*
13.00 -	**SANDWICHES AND GOODBYES**

Workshop in Aquatic Animal Epidemiology and Surveillance, 4-5 June 2007

at the National Veterinary Institute, Technical University of Denmark, Copenhagen, Denmark

Niels Jørgen Olesen gave an introduction to the workshop, with attention to the scientific program and the booklet, which includes abstracts for all the presentations and the new Council Directive 2006/88 in print. Sigrid Cabot from the European Commission gave some information of the practicalities of the meeting. Britt Bang Jensen presented the International Society for Aquatic Animal Epidemiology (ISAAE), which is a non-profit organisation promoting the interest in aquatic animal epidemiology, via participation in meetings and congresses, together with providing information on training and tools for use in this area. The members of the steering group of ISAAE have organized the present workshop. Further information on the organisation and its mission and objectives is to be found on the webpage www.isaaepi.org. Application Forms for the organisation is available on this web page, and everybody with an interest in epidemiology and aquaculture is encouraged to join. The Steering group holds meetings every month using the web based program Horizon Wimba. Tuesday the 5th of June a lecture in "Diagnostic testing in infectious disease surveillance" by Marios Giorgiadis will be broadcasted and recorded using this program.

SESSION I: GIS AND MOLECULAR TRACING

Chair: Olga Haenen

The use of GIS for spatial analyses of spread of Gyrodactylus salaris

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Abstract: *Gyrodactylus salaris* has been recorded in 46 Norwegian rivers since 1975, and the parasite represents a major threat to Atlantic salmon stocks. *G. salaris* has a restricted tolerance to salinity (Soleng & Bakke 1997). However, clustering of infected rivers that drain into shared fjord-systems suggests that a common route of inter-river transmission is by migrating infected fish in the fjords (Johnsen & Jensen 1986). Such dispersal raises the expectations that inter-river transmission should depend on the volume of freshwater inflow to fjords, and distance between river outlets.

This study presents empirical data on the distribution of *G. salaris* infected rivers in space and time in Norway. Infected rivers were categorized as either primary infected rivers or secondary infected rivers. Primary infected rivers were the first rivers in different fjord-systems in which *G. salaris* was recorded. An inter-river dispersal model, with postulated dispersal pathways through fjords is proposed. Dispersal initiates from primary infected rivers and continues to secondary infected rivers, from the nearest infected river in space and time. A logistic regression model was used in which secondary infected and non-infected rivers in shared fjord-systems were entered as the dependent variable, and independent geographic risk factors were tested for association. Freshwater inflow to the fjords was derived from the database Regine (Norwegian Water Resources and Energy Directorate), and we used Spatial Analyst (ESRI, Redlands, CA, USA) to compute relative freshwater inflow covering the postulated dispersal pathways. Pathway distance was measured as the shortest fjord-wise path between river outlets.

27 secondary infected and 55 non-infected rivers at risk were entered into the logistic regression model. There were significant effects of relative freshwater inflow to the postulated dispersal pathways and fjord-wise dispersal distance on the probability of secondary *G. salaris* infection. This is in accordance with predictions arising from the hypothesis of dispersal pathways of *G. salaris* on infected fish through fjords. It is argued that *G. salaris* is capable of dispersing to succeeding rivers in fjord-systems, but that such dispersal eventually will be

prevented by a combination of low freshwater inflow and long distance between river outlets. We propose that the present model framework can be used to map the risk of further dispersal of *G. salaris* in Norway.

References:

Johnsen BO, Jensen AJ (1986): Infestations of Atlantic salmon (*Salmo salar*) by *Gyrodactylus salaris* in Norwegian rivers. J Fish Biol 29: 233 – 241.
Soleng A, Bakke TA (1997): Salinity tolerance of *Gyrodactylus salaris* (Platyhelminthes, Monogenea): laboratory studies. Can J Fish Aquat Sci 54: 1837– 1845.

Notes from the presentation:

Peder A. Jansen presented the use of a Geographical Information System (GIS) for spatial analysis of *Gyrodactylus salaris* dispersal performed at the National Institute in Norway. The work with epidemiology was made on both farmed fish and wild fish. The question is: How to use GIS for modelling the spread of *G. salaris* in Norway? A model for *G. salaris* surveillance and example of knowledge-based advice has been implemented in Norway. *G. salaris* was introduced to a research fish farm in the early 1970's. Salmon juveniles were produced for cultivation and stocked in salmon rivers and there is a nearly perfect match between stocked rivers and where the parasite was originally found. *G. salaris* is a freshwater parasite in juvenile fish where prevalence of up to 100% and thousands of parasites per fish are common. The parasite is only a problem for salmon but there are many other carrier hosts. The reproduction of *G. salaris* is unique, in that it gives birth to maximum 4 offspring so the population may double in 3 days, and the potential for population increases with temperature. Looking at the distribution of *G. salaris* in Norway the parasite has been found in 46 rivers of which 15 rivers are considered cleared of infection due to the use of rotenone (poison that kills fish and parasites) indicating that the treatment has failed in many rivers. When Al solutions are used for treatment the parasites are killed but not the fish. There are surveillance programs for *G. salaris* in Norway.

G. salaris spread between rivers in the way that infected rivers drain into the same fjord system and non-infected rivers are not found in between infected rivers.

The model considered the probability of how the parasite spreads (p(spread)) through fjord systems with infected fish. P (spread) could be related to the following: freshwater inflow to fjords, distance between river outlets, the number of infected fish that migrates from infected rivers or the amount of time a river is exposed for infection

Other variables included in the original model were: The population size in source rivers, the exposure time and how long has the infection been present. The analysis resulted in the following final model: Logit (G. inf) = a + β_1 freshwater inflow + β_2 dispersal distance. The conclusion on the *G. salaris* dispersal indicates that there is good agreement between data and model. Given that the assumptions are right, the model may explain that all dispersal of *G. salaris* in Norway is due to: 1. Man moved infected fish and 2. Dispersal through fjords. There is a high risk of *G. salaris* dispersal. Furthermore the model has been useful in revising the Norwegian risk-based programme for surveillance and control of *G. salaris*.

Comments:

R. Rahkonen: *What about the sport fishing?*
P. Jansen: *The risk caused by sport fishing is very low.*
S. Helgason: *How is the risk when smolt from one river grows in another river?*
P. Jansen: *We do not know what happens but there might be infected fish, which goes between rivers.*

EPIZONE WP6.1: Surveillance and Epidemiology of emerging viral diseases in aquaculture – intentions and purpose

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Abstract: EPIZONE: Network of Excellence for Epizootic Disease Diagnosis and Control
The mission of EPIZONE is to improve research on preparedness, prevention, detection, and control of epizootic diseases within Europe. The procect consist of 4 vertical integration activities (themes): 1) Diagnostics 2) Intervention strategies 3) **Surveillance and Epidemiology** 4) Risk assessment.
The activities on aquaculture are placed in WP6.1 with focus on viral diseases, in particular generation of quantitative data and implementation of GIS, molecular epidemiology, emerging diseases and serology. The following topics are included:

- Generation of quantitative data
- Implementation of GIS
- Molecular characterization of virus isolates
- Establishment of a virus database.
- Koi Herpes Virus
- VHS & IHN antibody detection in fish

Due to special epidemiological conditions for fish farming knowledge on the de-facto occurrence and spreading of the notifiable diseases VHS and IHN is crucial. In the new Council Directive 2006/88/EF all farms shall in future be registered and their geographic position and health status informed. This will give us much better tools for obtaining that kind of information.
A virus database is under development and will enable possibilities for detailed molecular epidemiological analysis of the diseases. Fast tracking and vigilance towards new emerging diseases is important also in aquaculture, as an example Koi Herpes Virus has been included and the WP will provide data on the epidemiology and the diagnosis of this disease.
Finally the possibility of using serology in aquaculture as an alternative or supplement to virus identification will be assessed.

Notes from the presentation:
Niels Jørgen Olesen, who is the work package leader for workpackage 6.1, presented the Network of Excellence for Epizootic Disease Diagnosis and Control (EPIZONE). The project is founded under FP6 for 5 years and many diseases such as Avian Influenza, Vesicular Swine Fever and Foot& Mouth Disease and also notifiable fish diseases are included in the program. The mission of the project includes: Research on epizootic diseases including preparedness, prevention, detection and control via increased excellence by collaboration. An introduction to the different teams in EPIZONE was presented, with special attention to team 6.1; surveillance and epidemiology of emerging viral diseases in aquaculture. The main focus of this work package is the development of a database for molecular tracing of disease outbreaks. Topics such as research update on KHV and serology on VHS and IHN are also milestones included in the WP 6.1.
A map demonstrates the spread of VHS and IHN in Europe with an example of a map from Germany showing that 575 farms are under surveillance but as many as 3259 farms have an unknown disease status. N. J. Olesen points out that VHS and IHN are under-reported in the EU.

The new Council Directive 2006/88/EF demands that all farms are registered (licensed) and their geographic coordinates informed, together with their health status. In case of disease outbreaks of VHSV this information should be made available.
An example from Denmark was giving, indicating how coordinates from VHSV outbreaks in Denmark has been mapped by the use of Google Earth which is free software. For combining epidemiology with molecular tracing a large number of Danish isolates has been sent to Cefas for sequencing with the purpose of tracing the origin of the first VHS outbreak that occurred in England in 2006.
The work of developing a database for standardized isolate information for molecular tracing including GIS and sequence data has been initiated.

Comments:
B. Oidtmann: *The excellence of this database is a question of confidentiality.*
N. J. Olesen: *All published data will be available and the database should be open including the geographical coordinates but the names of the fish farms will be covered. Within the EPIZONE a database called GISAID DB has been made for Avian Influenza and all data in this database are confidential for 6 months with special reference for publication. Within our field scientists are collating lots of data, which is a gold mine of information, but the publication time is slow. Yet there is a need for sharing data to enable molecular tracing.*
B. Hjeltnes: *Regarding to confidentiality all information should be known for the authorities in Norway.*
N. J. Olesen: *This is correct for notifiable diseases – but within the DB the information will be very detailed.*
R. Rahkonen: *In Finland it is not permitted to publish and spot on the map but a directive says to use GIS if possible.*
S. Cabot: *Transparency is the future.*
B. Hjeltnes: *At the National Institute in Norway data and sensitive information is available for the public but not on a web site.*
N. J. Olesen: *We need as much transparency as possible.*

A proposal of a GIS data model to support the aquatic animal disease surveillance

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Abstract: Diseases surveillance and reporting is considered to be a fundamental part of any national or regional strategy on aquatic animal health. As result, there is a growing interest in developing better system for exploring and reporting of aquatic animal diseases and, in particular, documenting freedom from diseases. A geographic information system (GIS) offer such a wide useful range of capacities that they should be incorporate into all aquaculture surveillance system both for general aquaculture management and veterinary surveillance. Stream, catchments and basin, organised according to the Arc Hydro data model, can be combined with fisheries location, epidemiological data and water resource data inventories to create a veterinary hydrologic information system. This information system can be used to support contingency planning and monitoring of diseases control measures, to provide of sound aquatic animal health advice to farmer, to produce certification of exports, international reporting, confirmation of freedom from diseases and assurance of pathogen status and finally for an effective aquatic animal emergency diseases readiness.
The core of the system is the integration of the "new" feature fish-farm into the Arc Hydro data model. A hypothesis of the geometry of the spatial object, its spatial relationship, its topological

relationship and the methods, properties and events associated with this new object are presented.

References:

Cameron A. (2002): Survey Toolbox for Aquatic Animal Diseases: A Pratical Manual and software
package. Australian Centre for International Agricultural Research. Canberra, Australia
ESRI (Environmental Systems Research Institute, Inc.) (1992): Cell-based modeling with GRID 6.1. Hydrological and distance modeling tools. ESRI, Supplement, Redlands, California.
ESRI (Environmental Systems Research Institute, Inc.). (2001):. ArcGIS Hydro Data Model (Draft,
July 2001). ESRI, Technical paper, Redlands, California.
Fisher, W. L., F. J. Rahel (2004): Geographic Information Systems in Fisheries. hardbound Published by American Fisheries Society Publication. USA
Maidment, D.R. (2002): Arc Hydro: GIS for water resources. ESRI press. Redlands California.
OIE (World Organisation for Animal Health) (2006): Manual of Diagnostic Test for Aquatic Animals. OIE. Paris
Tomlinson, R. (2003): Thinking about GIS, Geographic information system planning for managers.
ESRI press. Redlands California.

Notes from the presentation:

Nicola Ferré presented a proposal of a GIS data model to support in aquatic animal health surveillance. GIS is the acronym of Geographic Information System, which is understood as a system for capturing, storing, checking, manipulating, analyzing and displaying data which are spatially referenced to the earth (Clorely 1987).

One of the main characteristics of GIS is the possibility to join the geographical information with the attributes: data that describe the geographic object. Example of attributes are: name of the fish farm, number of fish, species, test results, etc. The attributes can be filtered and used for creation of different maps showing different information. By means of the interoperability, the source of this data (the data server) can be distributed all over a network either at enterprise level or Internet level.

Generally speaking, GIS applied in veterinary activities, considers the geographic event as object distributed in a “Euclidean Space”. In this kind of assumption, the relationship between the events depends only on the distance (stationary process) between the features. But when we speak about the fish farm, this assumption is not valid at all: the relationship between fish farms must be considered in term of network analysis. Only by taking into account the stream network is it possible to perform a hydrology analysis.

A suitable and well-established data model for the hydrology analysis is the ESRI ArcGIS Hydro (or Arc Hyrdo) which provides a framework for organizing and preprocessing geospatial and temporal data in a GIS for use in hydrologic and hydraulic models. Unfortunately the fish farm object is not already developed into the Arc Hydro framework. This constrain can be overcome by creating a relationship of the fish farm object with the system objects already developed by the framework (water withdrawel and water discharge points). Once the fish farm object will be tied into the Arc Hydro framework several analysis are possible like the network navigation, the next downstream navigation, the river addressing and the drainage analysis. All these analysis can be very useful for the aquatic animal health surveillance activities.

Ferrè proposed the establishment of a working group among the participants for the analysis and implementation of the fish farm object into the Arc Hydro framework. This working group must take into account the constraint introduced by the EU Directive 2007/2/EC, the INSPIRE directive (INfrastructure for SPatial InfoRmation in Europe, **http://inspire.jrc.it/**).

In the near future Nicola Ferrè and Giuseppe Bovo will implement a Web-GIS test platform that could be used to test the integration of fish farm objects into the Arc Hydro framework. All the

273 fish farms in the Veneto region will be tied into the framework and the results of the GIS data model to support in aquatic animal health surveillance will be presented.

Nicola Ferré is willing to share knowledge and can be contacted by mail:
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Comments:
P. Jansen: *Which streams are interesting rivers?*
N. Ferré: *Start by drawing a map only including a few farms for the test model with the purpose of exchanging knowledge.*
N. J. Olesen: *How do you register the fish farms?*
N. Ferré: *The location and health unit have the Y, X coordinates like the ones found in Google Earth and then information on the farms is registered.*

The first occurence of Viral Haemorrhagic Septicaemia (VHS) in England

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Abstract: In May 2006 a fish farmer in Yorkshire, England reported abnormal mortalities of rainbow trout on his farm. Rainbow trout were the only species held on site and were produced exclusively for the table market. The mortalities had initially started in late March 2006, and two private fish health specialists had examined the stock and failed to identify the causative agent. The fish had failed to respond to therapeutic treatment for suspected bacterial infection. The fish farm had been visited by the Fish Health Inspectorate in early March 2006 and a sample of fish taken for routine testing for List II diseases as required under EC Directive 91/67/EC. This test was negative for VHS.

On 22 May 2006 a Fish Health Inspector examined the fish farm stocks and observed clinical signs of disease. Samples were taken and a range of diagnostic tests was undertaken at the Cefas Weymouth laboratory. Samples of fish tissue comprising brain, spleen and kidney were inoculated on to the following cell lines, BF-2, CHSE-214, and EPC, and after 3 days incubation at 15ºC a cytopathic effect was observed. An ELISA test gave a presumptive positive result for VHSV and this was confirmed using an RT-PCR assay and sequence analysis. VHS was confirmed on 26 May 2006 and this was the first confirmation of VHSV genotype 1a in the UK.

A National Control Centre (NCC) was immediately established at Cefas Weymouth to implement the VHS contingency plan, and subsequently met regularly to co-ordinate the control measures designed to stamp out the disease. Fish Health Inspectors immediately undertook a humane cull of the remaining fish stocks on site, with the dead fish removed to a government-approved animal waste processing plant for rendering and incineration. The fish farm was emptied of stock and drained of water by 28 May 2006. The fish farm was subsequently disinfected and fallowed.

On confirmation of VHS, approved zone status for UK was suspended, and statutory controls were placed on the index site, and all 33 fish farms in the catchment. Contact testing was undertaken on sites that had supplied fish to the affected farm, and a surveillance programme involving sampling of fish farms in the catchment was conducted. In addition an extensive wild fish-monitoring programme was conducted.

Following a series of negative tests of fish farms in the affected catchment, statutory controls were varied to allow movement of live fish out of the affected area. However controls were maintained on farms located within proximity of the index site. The catchment has since been subject to an intensive surveillance programme on both farms and in wild fish.

Following an extensive epidemiological investigation, a definitive source of infection was not identified. The epidemiological investigation is continuing. Current evidence indicates that the disease has been eradicated from the UK.

Notes from the presentation:
Great Britain has long-standing fish health controls, originating with the 1937 Diseases of Fish Act, which prohibited the import of live salmonids into the country. In addition VHS has been a notifiable disease since 1973. With the introduction of the single market measure EC Directive 91/67/EEC Great Britain was recognised as an approved zone for VHS on the grounds of historical freedom from this disease. With the exception of a single outbreak of a marine genotype of VHSV in turbot in 1994, over 15 years surveillance on fish farms in mainland GB has shown no evidence for the presence of the disease.

In May 2006 the Cefas Fish Health Inspectorate was notified of persistent mortalities on a fish farm in North Yorkshire. Mortalities had initially started in March 2006 but fish health consultants had failed to identify the causative agent. VHS was confirmed on 26 May, the fish farm was immediately subject to official controls, and the stocks culled. The site was then disinfected and fallowed. As a precautionary measure all 33 fish farms in the river catchment were placed under statutory control, and two rounds of sampling for VHS undertaken on each site. These tests proved negative for VHSV. Subsequently regular inspections have been conducted in the catchment with no evidence for VHS on any of the farms.

To investigate whether there was any reservoir of infection in the catchment, extensive sampling of wild fish was conducted both upstream and downstream of the infected fish farm. Some 308 fish comprising grayling, brown trout and pike were tested for VHS. Immediately following the confirmation of VHS on the fish farm, 1 grayling downstream of the discharge point was found to be infected. All other tests have subsequently proven negative. Wild fish monitoring in a buffer zone downstream of the normal tidal limits of the catchment is continuing.

A thorough epidemiological investigation into the disease outbreak was conducted. However a definitive source of the infection has yet to be identified. Current evidence indicates that the VHS outbreak was a single event affecting 1 fish farm, and that the disease was eradicated by prompt stamping out of the infection.

The 2006 Viral Haemorrhagic Septicaemia Outbreak in England– Epidemiological Investigation into the origin of infection

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Abstract: In May 2006, VHS was diagnosed for the first time in England. The only other recorded VHS outbreak in the UK was in a land-based turbot farm in Scotland in 1994.
A thorough investigation was launched in order to identify the source of the outbreak, to identify other farms that may have been affected by the same route of infection, prevent further introductions, and contain the outbreak as quickly as possible.

After an initial assessment, no obvious route of introduction could be identified. Generic scenario trees were drawn up to identify all possible routes of introduction of VHS strain 1a into the UK. The applicability of each pathway for this particular outbreak was then evaluated.
Pathways of relevance for the case were investigated further. Pathways investigated included live fish movements, movements of fish products, fish waste, mechanical transmission and other routes, such as wild fish. Due to the health status of the UK prior to the outbreak (i.e. free from VHS), live fish of the susceptible species could not legally be imported. However, illegal live fish movements are a theoretical pathway. Further potential pathways identified include movements of fish products and mechanical transmission.
The source of the outbreak has not been identified to date. Increased mortalities on the affected farm were reported to the competent authorities with some delay, which may have impaired the chances of establishing the source. No further outbreaks have occurred, suggesting that the introduction was via an unusual pathway.

Notes from the presentation:
The infected farm is divided into 3 separate units (A, B, C). Fry was brought onto the site (unit A) on the 23rd of March 2006. Mortalities started at the end of March in unit C, which receives second use water from the A and B units. During April 2006, increased mortalities spread to further raceways of unit C and to unit B. The mortalities first increased a week after fry were brought onto site, suggesting the fry to be the source of infection. The fry supplier had received eggs from Denmark and had mortalities on site in November 2005. In addition, the fry supplier had not kept mortality records between January and May 2006. However, the fry received from the supplier were unaffected until one month after delivery. The fry supplier was hence investigated; fish sampled tested negative.
The time window of entry was estimated based on data by Neukirch (1990); Neukirch reported the median survival time of rainbow trout exposed to VHSV at high exposure dose and 4ºC to be 16 days. Based on those data, the introduction point would have been up to 70 days before noticeable increase of mortality. However, since there is insufficient data about how long VHS may stay sub clinical at low temperatures and given that the infective dose may have been substantially lower at the affected farm, the time window could be longer.

Potential pathways of introduction, such as movement and stocking of live fish into the river, fish waste and fish products in coherence with a smokery or processing site were investigated. But also mechanical transmission via live fish transporters, transport vehicles used for fish for processing, fish waste movements, birds, angling etcetera were considered. In wild fish sampled shortly after the outbreak, VHSV was isolated from a pool of seven grayling out of 120 fish tested in the week after VHS was identified. .

So far, it has not been possible to establish the source of infection. Factors that have made establishing the source difficult are: limited availability of comprehensive mortality data, significant knowledge-gaps regarding VHS, incomplete or inconsistent information provided by the industry (making interpretation difficult) and the late reporting of unusual of mortality at the trout farm to the competent authorities. Furthermore documentation of the trade of fish products from European sources is not freely available and tracing imports of potentially infected fish products from abroad is difficult.
The VHS outbreak in England in 2006 might be a result of a combination of an unusual and improbable sequence of events.

Comments:
H. Hellberg: *Why did the private fish health experts (who were initially consulted by the fish farmer) not investigate earlier for presence of VHSV?*

B. Oidtmann: *It might be because there was no history of VHS in England. Therefore, private fish health experts would have normally looked for bacterial diseases and there were no classical signs of VHS.*
B. Hjeltnes: *Did the private fish health experts take any samples for virology?*
B. Oidtmann: *Not in the early samples taken.*
R. Rahkonen: *We have tried to learn how VHS looks like but have experienced that the Veterinarians take samples from fish with no clinical signs. The time window might be very little and it is a matter of catching the right fish. Maybe there is a problem with the diagnostic tests available.*
O. Haenen: *The confirmation was made in cell cultures but why didn't you use PCR techniques?*
K. Denham. *Initial isolation was made in cell culture, and the virus was confirmed by ELISA and PCR in the cell culture harvest. As VHS had not previously been found in mainland UK, and the clinical signs of disease were not typical of VHS there was nothing to indicate that PCR should have been used on the sample material.*
N. J. Olesen: *Blood samples could have been taken before you culled the fish to test for antibodies. Furthermore our infection trial shows that the VHS isolate from England cause higher mortality than our positive controls.*
R. Raynard: *In Scotland, molecular techniques have been used and quite a lot has been done but the origin has not been identified.*
G. Bovo: *At low temperatures there is no sign of disease but when the temperature rises the mortality appears which often is in late April or May.*
B. Oidtmann: *The presentation of a VHS outbreak can vary substantially depending on host strain (e.g. rainbow trout), and viral VHS strains.*
S. Bergmann: *Have you tested other species?*
B. Oidtmann/K. Denham: *Yes. A number of species of wild fish from the river catchment were tested for VHS following the disease outbreak.*

SESSION IIA: RISK BASED SURVEILLANCE

Chair: Brit Hjeltnes

The New Aquatic Animal Health Directive on Surveillance and its Implementation

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Abstract: The new aquatic animal health Directive (Council Directive 2006/88/EC on animal health requirements for aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals) was adopted 24 October 2006. The Member States shall adopt their implementing legislation before 1 May 2008 and apply these from 1 August 2008.
The Directive contains several provisions on surveillance and disease notification:

- Article 7 requires Member States to conduct official controls in accordance with the Control Regulation (EC) No 882/2004.
- Article 10 requires a risk-based animal health scheme to be applied in all aquaculture farms and farming areas. This surveillance may be conducted by the competent authority or a qualified animal health service. The aim is to detect any increased mortality and the

diseases listed by the Directive. Recommendations on the inspection frequency are laid down in Annex III Part B to the Directive.

- Article 26 obliges any person with an occupational relationship to aquatic animals of susceptible species or to products of such animals to notify suspicion of any listed diseases or increased mortality to the competent authority of the Member State.
- Article 44, 49 and 52 sets up the basic requirements, including procedural provisions, regarding the adoption of surveillance and eradication programmes and the surveillance to maintain disease freedom in Member States, zones and compartments.

The Commission intends to draw up the following implementing documents in relation to surveillance:

- Guidelines on risk categorisation of farms.
- Commission Decision on the sampling plans and diagnostic method for:
  1. The detection and confirmation of the listed diseases, and
  2. The targeted surveillance to be carried out to obtain and maintain disease freedom in a Member State, zone or compartment.

Notes from the presentation:

Art 26: Concerns Notification: All farms have the duty to report immediately to the competent authorities (CA) when there is suspicion of a listed disease. They should notify the CA or a private veterinarian in case of increased mortality. This obligation lies on: fish farmer, transporter, veterinarian or any person with occupation on susceptible species.

Listed diseases:

	Exotic	Non-exotic
Fish	EHN	SVC
	EUS	KHV
		VHS
		IHN
		ISA
Molluscs	*Bonamia exitiosa*	*Marteilia refringens*
	Perkinsus marinus	*Bonamia ostreae*
	Microcytos Mackini	
Crustaceans	Taura syndrome	White spot disease
	Yellowhead disease	

According to the directive, increased mortality: ”means unexplained mortalities significantly above the level of what is considered to be normal for the farm or mollusc farming area in question under the prevailing conditions”. What is considered to be increased mortality shall be decided in cooperation between the farmer and the competent authority”

Art 7: Official controls are carried out by the CA and involves regular inspections, audits, and where appropriate, sampling. The official controls should take account of contracting and spreading of disease and other risk factors as mentioned in regulative (EC) 882/2004 art. 3. Recommended frequencies of control visits are stated in annex III of 2006/88/EC.

Art 10: The animal health surveillance scheme includes all farms, and is carried out by the CA or qualified aquatic animal health service. A risk-based animal health surveillance scheme as appropriate for the type of production should be aimed to detect any increased mortalities and listed diseases. Frequencies for visits as found in annex III regarding art. 7 and 10 may be combined so one visit may be used for both purposes if appropriate.

Art. 32/39: Describes containment measures that should be applied in case of confirmation of a listed disease. A containment area is ”an area around an infected farm or mollusc farming area where disease control measures are applied with the purpose of preventing the spread of the disease” The containment measures to be taken are: Declare farm infected, establish containment area including a protection and surveillance zone appropriate to the disease in question, restrict movement out of the containment area and so forth.

Surveillance and eradication programmes; The targeted surveillance for achieving disease freedom as described in article 44, 49 and annex V will probably not be risk-based.

Art 52: Maintenance of disease freedom:
Targeted surveillance may discontinue where the whole MS is disease free. Targeted surveillance at a level commensurate with the level of risk can be applied in disease free zones and compartments or when conditions conductive to clinical expression do not exist.

Annex III part B indicates in which category the farms should be. The table is relevant for each disease, not for each farm. A farm as such might be in different categories, if the disease status as regards the listed diseases differs. A farm may be free of one disease (cat. II), under surveillance for another (cat. II), and infected regarding a third (cat. III).
Commission implementing measures includes i.e. guidelines on risk categorisation of farms and a Commission decision on sampling plans and diagnostic methods for detection and targeted surveillance. Commission decision 2001/183/EC and 2003/466/EC will serve as a template and the OIE code will be used as a basis when appropriate.

Art. 54: “Each member state shall ensure that the CA has access to adequate laboratory services and state-of-the-art know-how in risk analysis and epidemiology”

Commission guidelines on risk categorisation on farm should be practicable and workable and be general enough to take into account the variation of the industry, and be as unambiguous as possible to aim at a harmonised approach in the community.

Factors for risk categorisation of farms might be divided into:
-Risk of introduction of a disease agent from farms/wild animals:
- -species kept(susceptible/vectors)
- -conditions conducive to clinical expression of the disease
- -type of farm and hygienic status
- -geographical position
- -animal supply/delivery

-Risk of spreading a disease agent to other farms:
- -As above

-Consequences of spreading a disease agent to other farms:
- -factors to potential receiving farms
- -the probability of being able to control an outbreak/eradicate the disease if infected.

Comments:
B. Hill: *When a farm is declared free but at high risk it is a potential danger, so perhaps a high-risk farm should not be declared free. So biosecurity is a very important factor. Deal with the risks –i.e. increase biosecurity.*
S. Cabot: *If the member state is free, the risk will be low, but still some farms are at higher risk if they for example import fish from other countries.*
P. Østergård: *The sampling is very infrequent.*

B. Hjeltnes: *These are just recommendations, and the frequency could be increased. But you cannot tell the management of the farm that they cannot jeopardise their farm. The biggest risk is transport of biological material but that is against free trade.*
S. Bergmann: *There is one risk for farm and one risk for disease introduction.*
B. Oidtmann: *Could other farms get access to the risk category of the farm with whom they want to trade, so that the farmer can choose if he accepts this risk? That is a problem for lower stream farms.*
S. Cabot: *It is required that each Member State establishes a database, which will include information on the disease category of the farm of all the listed non-exotic diseases. It will probably not be required to give information on the risk categorisation of a farm.*
R. Rahkonen: *The directive is for authorities, but the farmer can demand what he wants – perhaps more restrictions.*
N. Ferré: *How will article 59 be handled?*
S. Cabot: *A draft on how this should be handled has been written and been discussed with the MS. The draft is not yet finalised and we are pleased to receive any comments.*
G. Bovo: *The CA can do the sampling for both article 7 and 10 on the same time, which means the sampling will be halved and the inspection frequency lowered.*
S. Cabot: *Art. 7 is flexible and should be determined in each member state, the guidelines given are only recommendations.*
N.J. Olesen: *there are several goals in this directive; one is to make sure you can have safe and trustworthy trade. But how is it supposed to be carried out –does each MS have to make their own scheme on how to carry out RBS, and how can we then trust them?*
S. Cabot: *The MS have to report how they implement the directive in their legislation to the Commission by may 1st 2008, and the Commission will assess this. The establishment of a risk based surveillance scheme under art. 10 is compulsory.*
R. Rahkonen: *There is a separate commission decision on how to achieve status of freedom from disease. And the MS have to present a declaration to the SCFCAH (Standing Committee on the Food Chain and Animal Health) on disease free zones and compartments comprising 75 % of the territories. Larger areas and whole disease free Member States need to be approved by the Commission.*
Kvellested: *How can you consider health of farm one by one when they are close together?*
S. Cabot: *How to manage the risk of farms located close together should be decided by the Member States. The Commission guidelines might give some guidance on how this might be done.*

What is risk-based surveillance and how does it apply to the control of fish health?

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Abstract: The new European Fish Health Directive 2006/88 (FHD), to be implemented on 1 August 2008, requires that Member States apply risk-based animal health surveillance. Some elements of the concept are already applied in current aquatic animal health surveillance schemes. Stärk et al. (2006) recently reviewed the current concepts of risk-based surveillance. Based on this review, the terminology and background to risk-based surveillance is explained and its application to aquatic animal health surveillance discussed.

The aim of risk-based surveillance is to allocate available resources effectively and efficiently and to improve the benefit-cost ratio compared with traditional disease surveillance. Risk-based surveillance uses a risk assessment approach to identify surveillance priorities and to select

high-risk groups of animals or farms. To date risk assessment in aquatic animal health has mainly been used to assess the risk of disease introduction with the importation of live animals or their products (import risk analysis – IRA) and thus to justify trade restrictions Guidelines for IRA are provided by the OIE (OIE 2006). However, the risk assessment approaches can also used to identify the most relevant diseases for surveillance and control on a national or international (e.g. EU) level. Annex IV of the FHD provides the criteria for selecting the diseases listed (for the purpose of control in the whole or parts of the EU) which are predominantly-risk based. A homologous approach could be taken at the level of individual Member States, which may consider controlling further diseases not currently listed by the FHD. In addition to the higher-level strategy decisions (prioritisation of diseases), risk-based surveillance aims to make best use of resources at an operational level. This could include targeting certain population strata (for example size or developmental stage) that are at a particular risk of being infected with a certain pathogen. Such population strata can be constructed depending on epidemiological risk factors that have been identified in previous studies. Such risk factors may include:

- Susceptibility of a certain age group of animal
- Introductions of animals into the population
- Water supply (potential exposure of an aquaculture production business to pathogens in the supplying water)
- Species farmed
- Biosecurity on aquaculture production business
- Processing unit on site processing aquatic animals from other aquaculture production businesses.

The question of how to compare the equivalence of different risk-based surveillance schemes needs further investigation. This is important to avoid disputes in international trade. Risk-based surveillance schemes therefore need to be objective and transparent.

Notes from the presentation:

The terminology of Risk based surveillance was explained firstly by giving examples of definitions of the terms risk and surveillance.
Risk analysis is the probability of occurrence of an undesired event and the consequences or costs of this event (International Animal Health Code, OIE 2006)

The definition of surveillance, based on the OIE Aquatic Animal Health Code (2006), is: "a systematic series of investigations of a given population of aquatic animals to detect the occurrence of disease for control purposes, and which may involve testing samples of a population."

Risk based surveillance (RBS) as defined by Staerck et al. (2006) is:
"A surveillance programme in the design of which exposure and risk assessment methods have been applied together with traditional design approaches in order to ensure appropriate and cost-effective data collection"
This employs strategic decision-making and optimisation of sampling at an operational level

The aims of risk-based surveillance is to identify surveillance needs, set priorities and allocate resources effectively and efficiently
Targeted surveillance is a part of RBS, and involves making best use of resources.

How to apply RBS to aquatic animal health:

1. Risk-based hazard selection
   Hazard identification
   - A list of relevant diseases and - commodities is provided in Directive 2006/88

The criteria for listing pathogens in the Directive are specified in annex IV, part I
The strategic decision as to whether or not to try to eradicate / maintain disease free status for listed non-exotic diseases is taken at Member State level.

Steps in risk assessment

2. Decisions at operational level.
   It has to be decided which methods should be used for surveillance, depending on the pathogen in question. Things to consider includes:
   Type of surveillance (passive, active, targeted)
   Frequency of sampling
   Selection of strata (pathogen prevalence may be higher in certain strata), surveillance will be directed by risk factors at farm level, (the risk levels are laid out in annex III, but these are rather vague; only for farms with undetermined disease status will RBS be compulsory by commission); risk factors within farms,
   Diagnostic tests (not yet defined, but harmonized at Community level –default OIE manual) –should there be flexibility?
   Sample size –Needs to be varied: factors such as expected prevalence and local circumstances need to be taken into account.

Many of the factors relevant for designing risk-based surveillance are also listed in the OIE guidelines for a release assessment of pathogens.

Summary: MS and 3rd countries need clarification as to what level of guidance will be provided by Commission to know what amount of surveillance planning will be required by the MS/ third countries.

Discussion: More details are needed in the new Directive, i.e. definition on what is increased mortalities, completion of the list of susceptible species, and what are the vector species.
The timelines are tight.
Diagnostic tests are not fit for purpose (cannot detect if no clinical signs), and it needs to be investigated whether these rules conflicts with the OIE-guidelines
How do we evaluate RBS-surveillance schemes?
RBS needs to be objective and transparent
Key for success: efficient disease notification
Benefit-costs ratio: Cost of implementing and maintaining RBS might be very costly

Comments:
B. Hill: *Should it be acceptable to allow movement of fish from high-risk to low risk farms with the same disease-free status?*
B. Oidtmann: *According to the Directive this is allowed, but there will be a requirement for a health certificate accompanying the movement.*
B. Hill: *If a low-risk farm imports from a high-risk farm, will the farm then become high-risk?*
B. Oidtmann: *I think there would be a good argument for it, but the Commission needs to consider specific examples.*
S, Cabot: *What is done today in disease-free areas?*
B. Hill: *Trade is restricted to that between zones with equal status, or from approved to not approved.*
B. Oidtmann: *Is it possible to run health control programmes for other diseases, not on the list?*
S. Cabot: *The MS can advocate for an additional disease to go on the list, and there is a flexibility in that the MS can take measures if they report to SCoFCA*
N. J. Olesen: *Many of us are unsure as to how this will be applied in the future. How much have you done in UK so far? And what were your thoughts?*

B. Oidtmann: *We have set down groups that are working with this, but are still awaiting news from the Commission – We hope to know more after this meeting.*
H. Hellberg: *RBS is not totally new; it is more that what we have been doing subconsciously in Norway is now written down. And we are applying all our previous experience.*
S. Cabot: *The Directive is building on what has been done before, and assumes that we have been taking RB approaches on a daily basis before.*

Diagnostic testing in infectious disease surveillance

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Notes from the presentation:
Diagnostic testing plays an important role in clinical and population health decision-making processes. Adequate tests are necessary for use in surveillance, and tests detect what they are designed to, but they sometimes make errors. Test evaluation is essential to provide estimates of the probability of errors of the diagnostic test under specific circumstances and for a given purpose. Test evaluation is easier for binary tests, which are used to find whether an animal has an infection or not, but it becomes very complicated when the tests have continuous outcomes.
These error probabilities are the (diagnostic) sensitivity and (diagnostic) specificity of the test.
Sensitivity is the probability that the test will identify correctly a sample from an infected animal
Se= Pr (T+|I+) (T= test, I= infection)
Specificity is the probability that the test will identify correctly a sample from a non-infected animal
Sp= Pr (T-|I-) If the test does not have 100% Sp, you can find test-positive animals even though there is no infection present. This is important in decision-making, and when planning surveillance and control programs.

Positive predictive value (PVP) is the probability that the positive sample comes from a positive animal. PVP = Pr (I+|T+). PVP only concerns animals that are T+, while Se only concerns animals that are I+.

$$PVP = \frac{\text{Pr (true positive)}}{\text{Pr (true positive)} + \text{Pr (false positive)}}$$

Negative predictive value is the probability that the negative sample comes from a negative animal
PVN = Pr (I-|T-).
The 2x2 table:

Test result \ True infection status	+	-
+	a	b
-	c	d

Se= a/(a+c)
Sp= d/(b+d)
PVP= a/(a+b)
PVN= d/(c+d)

The PVP and PVN depends on the prevalence of infection, meaning that if the same test is used on two different populations, the predictive values of the test could be different if the prevalence of infection is different in the two populations.
When testing low prevalence situations the specificity of the test should be close to 100% in order to minimise the false positive samples.

Factors that influence Se are errors in collection, transportation, storage and processing of the samples, stage of infection, severity of infection i.e. asymptomatic carriers, and differences in

the populations (natural, experimental). Factors that influence Sp are related to the occurrence of false positive due to cross-reactions, non-specific reactions or vaccination.

Test-evaluation is easy if there is a gold standard, but if not, there are statistical methods that can produce estimates of sensitivity and specificity in the absence of a gold standard, using free software i.e. from www.epi.ucdavis,edu/diagnostictests/

Serological test results are expressed as antibody titres based on successive dilutions or as continuous readings from a single sample – Interpretation of results is usually done with reference to a cut-off value. The selection of a cut-off value depends on the problem at hand; i.e. the purpose and setting of testing, the relative cost of each type of error and the existence of confirmatory test.
In screening we usually use a test with high se and then retest the positives with a test with high sp.
ROC (receiver operating characteristic) curves can be used to show how Se and Sp varies with different cut-off values, when looking for an adequate cut-off value. Area under the ROC curve can be used as a measure of the diagnostic performance of the test. A test with 100% Se/Sp will have an area of 1.

Comments:
N.J, Olesen: *Most of our tests are not binary. What do we do when our tests are not binary?*
M. Georgiadis: *It can be done, using statistical models, but it is difficult. But in decision making what you need is to know whether or not the disease is present.*
N.J. Olesen: *In aquaculture we do not look at single fish, but at herd level*
M. Georgiadis: *There exists methodology to produce estimates of sensitivity and specificity at the herd level*
H. Vigre: *If we have positive results, how should we interpret them in light of the low PVP in low prevalence situations?*
M. Georgiadis: *You have to be very cautious. When we test low prevalence populations with tests with imperfect specificity most of the positive test results will be false. This is something that can happen, for example, at the later stages of disease eradication programs.*
M. Georgiadis: *You can increase PVP by sampling high-risk populations (e.g. fish with clinical signs), but the point of risk-based surveillance is that you have to know what it is you are looking for.*

The new OIE guidelines for aquatic animal health surveillance

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Abstract: The OIE standards for aquatic animal health are presented in the *Aquatic Animal Health Code* (the Aquatic Code) and the *Manual of Diagnostic Tests for Aquatic Animals* (the Aquatic Manual). Currently, the Aquatic Code has no guidelines on disease surveillance, but in the Aquatic Manual there is a general chapter (Chapter 1.1.4) on 'Requirements for surveillance for international recognition of freedom from infection', and more specific chapters (Chapters 1.1., 1.2. and 1.3.) giving information for fish/mollusc/crustacean health surveillance and control programmes. However, these guidelines have remained mostly unchanged for several years and are in need of review and updating. It has also been decided to prepare a new Aquatic Code chapter on the general principles of aquatic animal health surveillance based on the equivalent chapter in the *Terrestrial Animal Health Code*. To achieve this, the OIE has convened an *ad hoc* group comprising four aquatic animal health specialists and epidemiologists

(the membership and terms of reference of this group will be presented) to advise the Aquatic Animal Health Standards Commission.

The ad hoc group has so far met twice and has drafted the new Aquatic Code chapter explaining the prerequisites and principles of surveillance, the main types of surveillance, the critical elements of surveillance, structured population-based surveys, structured non-random surveillance, surveillance to demonstrate freedom from disease/infection and surveillance to determine the distribution and occurrence of infection. The group has also substantially revised Chapter 1.1.4 of the Aquatic Manual giving more specific details of the procedures to be followed to demonstrate freedom from disease/infection, including guidance on the target population, sources of evidence to support claims of freedom, statistical methodology, clustering of infection, test characteristics and sample size calculation. The chapter also provides details of the specific requirements for structured survey design and analysis to assess disease occurrence. Examples are given for fish, mollusc and crustaceans to describe surveillance systems and approaches to the analysis of evidence for demonstrating freedom from disease, in order to illustrate the range of approaches that may be acceptable and to provide practical guidance and models that may be used for the design of specific surveillance systems. These draft chapters have been sent to all 168 OIE member countries for comments from their experts, and the European Commission will hold a meeting to co-ordinate the views of EU Member States before submitting comments to the OIE by 6 August 2007.

The OIE ad hoc group is also preparing additional specific guidelines to account for the differences in requirements for surveillance for fish, mollusc and crustacean diseases, as well as guidance to the authors of the disease chapters in the Aquatic Manual for specifying the surveillance requirements for each individual disease. These will also be distributed widely for comment. Ultimately, all the guidelines will have to be approved by OIE member countries before they can be adopted.

Some of the key elements of the proposed guidelines will be presented and discussed.

Notes from the presentation:
These guidelines have been prepared by an OIE Ad Hoc Group and are still at the draft stage waiting for OIE member countries comments.
It is important to note that the guidelines are for the whole world, so they need to be useful for every country and therefore are very detailed. Especially, there are detailed instructions on how to sample and the sample sizes to be taken. If there is a gold standard for the diagnostic test, the sample size can be calculated using FreeCalc, but if sensitivity and specificity are not known, there is a table in the guidelines giving the default sample size based on assumption of 100% sensitivity and sensitivity.

A farm that is considered free may discontinue targeted surveillance, if they adapt proper bio security measures continuously. These bio security measures are also defined in the guidelines.

The general guidance chapters are like a textbook, giving explanations on terminology in surveillance and epidemiology. The guidance does not use risk ranking of farms but is aimed at surveillance to demonstrate freedom from disease or surveillance for distribution of disease. Examples of surveillance programmes for fish, mollusc and crustacean diseases are given.

The OIE Ad Hoc group welcomes comments on the draft guidelines!

Comments:
B. Oidtmann: *If the OIE surveillance scheme is not adopted by the EU, will all the work have been in vain?*
B. Hill: *Not really – it isn't just for EU use, and in any case the EU MS will have approved them at the OIE so presumably will apply them. We will see when we get the OIE specific guidelines for surveillance of the individual diseases listed in Directive 2006/88.*
S. Cabot: *There is a working group meeting on June 19th where these guidelines will be discussed.*

Implementation of risk-based sampling methods and sample size in the surveillance to document freedom from IBR in the Danish cattle population

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Abstract: The presentation will focus on risk-based selection of sampling strata and sample size. The methods will be worked out using the evaluation and improvement of the traditional IBR surveillance in Denmark. IBR is defined, as infection with Bovine Herpesvirus in cattle and the disease is notifiable. Traditionally, the national surveillance program for IBR was based on 4 annual samples of bulk tank milk from dairy herds and sampling from every 6th slaughtered cattle. The assessment of this surveillance system was approached from two perspectives - relative to the international requirements for declaring freedom from disease and relative to the national needs for rapid detection of infected herds. The two approaches for the assessment primarily differed in the time frame that was used to estimate the surveillance sensitivity. The assessment involved the determination of the sensitivity of the surveillance system for detecting infected herds using different sampling scenarios. The surveillance for international requirements included all serologic testing of slaughter surveillance samples and bulk tank milk samples for a full year, whereas the surveillance for national needs was based on shorter time periods (1-3 months). Another difference between the assessment approaches was the prevalence of the disease. The international requirements dictate that a country should be able to detect at least 0.2% herd-level prevalence, whereas the threshold for the national needs was set to a single infected herd. The assessment was done for dairy and beef herds separately.

Based on existing estimates of test sensitivities on bulk tank milk, the sensitivity of the bulk tank milk surveillance during a year was estimated to nearly 100%, indicating that there is no need to include slaughter surveillance information for dairy herds to fulfil the international requirements. Based on existing estimates of test sensitivities on individual animals and utilisation of simulation, the sensitivity of the surveillance system in the beef population for a year was estimated to nearly 100%.

The surveillance to meet national requirements was evaluated under the traditional sampling conditions and three alternative sampling schedules (risk based selection of sampling strata and sample size). Modelling, including within-herd prevalence in infected herds and test sensitivity, demonstrated that the efficiency of detecting an infected dairy herd, which depends on bulk tank milk testing, would not be substantially decreased if the slaughter surveillance component was dropped. The efficiency of detecting an infected beef herd could only be improved by increasing the number of herds tested. Modelling showed that increased sampling-frequency of herds in high-risk areas could increase the likelihood of detecting disease.

Based on the results from this evaluation, the IBR surveillance has been adjusted to improve the efficiency of the system. The slaughter surveillance component has been dropped from the surveillance of dairy herds, whereas the frequency of sampling bulk tank milk from high-risk areas has increased. From dairy herds that have purchased imported animals the frequency of

bulk tank milk samples are increased for half a year. From beef herds one sample per herd per month should be collected at slaughter. From beef herds that have purchased imported animals two samples per herd per month should be collected for half a year. Following this sampling schedule, given that a beef herd has animals slaughtered, the herd will be tested every month. The adjustments of the traditional sampling (every 6th slaughtered animal tested), have reduced the number of serological testing at slaughter from ~120.000 to ~40.000. To enhance the efficiency for early detection of infected herds the sampling scheme was adjusted to focus on high-risk areas and herds. The high-risk areas and herds are identified using information on geographical location and occurrence of purchasing.

It is worth noticing that without knowledge based on a basic surveillance of the disease for many years, the risk-based adjustment of the surveillance system would not be possible.

Notes from the presentation:

The purpose of surveillance can be divided into international (i.e. trade) and national (i.e. control of outbreak).

The stages of disease occurrence versus surveillance are as follows: detection of disease ->eradication of disease-> prove freedom from disease-> detection of new outbreaks.

For Risk Based Surveillance (RBS) the efforts used for surveillance should harmonise with the probability and consequences of obtaining the disease. Therefore, formal evaluation of RBS programmes should be performed intermittently.

Infectious Bovine Respiratory disease is a Herpesvirus in cattle, which was introduced to DK in 1969. In 1980´es there were clinical outbreaks, and eradication was initiated in 1984, leading to declaration of freedom from disease in 1991.

From 1991 the surveillance was not targeted, since the risk was very high for the entire country. In 1995 there were 5 outbreaks, and the surveillance was changed to a risk-based one.

The evaluation of the surveillance was based on determination of the sensitivity of the surveillance program and it was approached from both an international (freedom) and a national perspective (detection).

The surveillance satisfied international requirements with gross margin.

The evaluation showed that for satisfying national requirements, some changes were necessary.

The sensitivity of the surveillance is increased when focus is on high-risk area and high-risk time periods.

In order to perform RBS, good knowledge of the disease is needed, and poor data leads to large uncertainties in the risk assessment and therefore a high risk for reducing the efficiency of the surveillance.

Concluding remarks: Make it simple to begin with and make room for adjustments based on experience. The success of targeted sampling depends on effective implementation of the sampling scheme.

Comments:

B. Oidtmann: *There are more possibilities for tests used in terrestrial animals. We often do not have sensitivity and specificity for the tests we use.*

R. Rahkonen: *It is no longer so fixed which tests should be used.*

H. Hellberg: *There is a fundamental difference in that you can not go back and re-test the animal in aquatic animals*

S. Cabot: *Would like to get comments from experts on whether we should do this RBS based on trial and error.*

B. Oidtmann: *The difference is that IBR is not listed by the EU, and the consequences of doing it wrong for the listed diseases are bigger.*

Demonstration on the UK live fish database

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Notes from the presentation:

The Fish Health Database includes over 500 registered aquaculture facilities and the data is managed trough a shared ownership board, while the ownership of the data resides with the originating body. Information is provided by importers, fish farmers, fishery owners, fishermen and the general public, and it is funded using government money.

The sharing of data is limited by data protection legislation and the access to the data is controlled via codes and permits, but will be open access in the future. There is already a limited release of generalised data through the website which is interactive, so people can apply for moving of fish etcetera.

In the database, each farm has a unique code, which is kept forever. There is a geographical code which can link to whatever Geo-reference system is required. For each farm, all species on the farm is listed, and the holding facilities, water source, processing plants, production, movement off and on and pollution incidents can be seen. GIS can be used to trace where the fish are moved and how they are distributed from there, since information from TRACES is incorporated. For each farm there is also status of disease, and results from diagnostic test and previous inspection visits. For each visit, the sample gets an anonymous number so the staff at the laboratory does not know which farm it is when they do the analysis. It is possible to search for all farms with e.g. SVC susceptible species, and to do queries for reports etcetera.

Comments:

B. Oidtmann: *Would like to know if other MS have a system in place?*
A. Kvellested: *In Norway there is a system for handling samples, which also include the diagnostics.*
B. Hjeltnes: *The fisheries have a database about production and feed used etc. There is a new system that should try and link loss in production with the diagnosis of diseases.*
S. Cabot: *The information you have is sufficient for risk categorization of farms. You are already applying some of the RBS in that you chose which farms to survey.*
B. Oidtmann: *The database can record mortalities, and some software could highlight when mortalities crosses a set threshold.*
R. Rahkonen: *Can the farmers see the data?*
C. Crane: *Some of them can, but they can not see everything. For example they can get their production data.*
B. Hjeltnes: *We need to keep in mind that we are serving different kinds of industries.*
A. Kvellested: *Geolocalization is very important, since the companies change names, owners and so on.*
S. Cabot: *The electronic register should be in place before august 2008.*

SESSION IIB: Examples of Practical applications of Risk Based Surveillance

Chair: Britt Bang Jensen

The scope of this Workshop Session was to give the participants an introduction to the concepts of risk based surveillance and how it can be applied practically. Birgit Oidtmann started with a presentation on one way to go forward with risk-based surveillance, as have been tried in a working group at Cefas.

This approach was for developing a program of risk-based surveillance for *Gyrodactalus salaris*.
The purpose is to risk rank river catchments and fish farms, and this could be done by first collating data in four areas (live farmed fish, wild fish, dead fish, mechanical transmission), followed by scoring using factors like: species farmed, number of contacts with other farms, does the farm receive eggs, total production of the farm, volume of live fish received and biosecurity measures on farms. Each factor is scored, and great consideration should be taken when deciding what should be the weighing factor for each risk factor. The final outcome is the risk for each farm of catchment, which can then be grouped into high, medium or low.
Potential consequences should also be taken into account.
Sigrid Cabot from the EC presented a table over factors important for introduction of disease, spreading etc. that is currently being used as a tool for the EC to decide which factors to consider and how.

The participants were divided into different groups based on geographical origin, assuming that similarities in geography also concurs similarity in production systems and species farmed. There were three geographical groups and one group discussing the use of passive surveillance. The groups had 1½ hour to discuss among themselves, and where then asked to present the outcome of their discussions for the entire workshop. The groups reported as follows:

Northern Europe:
(Reported by R. Raynard).
The focus for this exercise was on Infectious Salmon Anaemia, and the first half hour was spent on discussing the disease.
Salmon was ranked as a "high risk" for targeted surveillance, whereas rainbow trout was "low risk".
Risk classification: It was discussed that countries will have different approaches to risk categorization, a factor considered as "high risk" in one member state may be considered "low risk" in another.
Another factor to be considered is the intensity of production at a site, but probably movement of live fish and biological material is the highest risk.
With the current trend of centralisation, large farm networks are linked, together with processing plants, creating a dynamic industry that needs a system that is flexible and adaptable to these changes.
Finally, demonstration of freedom from ISA following an outbreak should be performed by targeted surveillance, depending on seasons.
The group had also had a short discussion on IHN which by most member states are considered as a high consequence disease.

A few general comments from this group were that Member States claiming equivalent status may not agree that their risk assessments are equivalent. Furthermore it could be claimed that targeted surveillance at imports classified as high risk could be considered a trade barrier.

Comments:
B. B. Jensen: *Did the group agree on these things, e.g. MS not performing risk assessments in the same way?*
R. Raynard: *It was raised as a thing that could give problems. These kinds of problems may emerge. Even thought two countries may be declared free of disease they may declare the same thing as low risk and high risk.*
B. B. Jensen: *It will be a huge task for the directive to give guidelines on this.*
S. Cabot: *If a MS sees something as a trade barrier they can go to Luxembourg with it.*

Eastern Europe:
(Reported by Caroline Crane)
The group chose KHV as the disease of focus. There was a lot of discussion on species susceptibility, especially dealing with what is a susceptible species and what is a carrier species. A bigger discussion was what would comprise the highest risk, the susceptible or the carrier species? A lot of the sites in Eastern Europe consist of large water bodies which are not drainable, making it difficult to find out what species are there. The risk that came out on top was movement of fish, which should be controlled by record keeping and documentation. Import was rated as the second highest risk. Mechanical transfer is also seen as a big risk, with veterinarians and others visiting different farms. Pet shops are another problem, with the ornamental fish trade going on very much uncontrolled. Finally the group discussed the problems of diagnostics and the disease will actually be found if it is there.

Comments:
O. Haenen: *It could be interesting to look at antibody levels to find out if the disease is present in those very large waterways.*
B, Oidtmann: *Another aspect was that due to the problems with diagnosing the diseases the Eastern European countries will be placed in the group with status unknown where there are no trade barriers which could mean that they will actually import the disease being in this group.*

Southern Europe:
(Reported by Giuseppe Bovo)
This group chose VHS as the focus disease and tried to follow the scheme provided by Sigrid Cabot, but there where a lot of discussions beforehand.
The group agrees that defining susceptible species is a fundamental thing and we need to learn from the recent outbreak from North America, which shows that it is hard to tell what a susceptible species is. If a vector species is considered as a mechanical vector, then there is plenty of species that can be vectors.
Since the disease is temperature-dependent and the temperature in the region can be very high 16-17 degrees year round, it means that the disease is not seen even though the virus is there. This gives a high risk of spreading the disease.
An important risk factor is application of biosecurity measures – farms not applying these are high-risk farms as farms could be at very high risk if basic hygienic measures are not applied.
Farms situated upstream from a farm is also a high risk.
The disease status is very important, but if the farm is approved free, it can still have some risk, for example if the farm is surrounded by non-approved farms, the risk would be high.
The number of previous outbreaks may be considered a risk factor, especially if the farm has not identified the source.
Proximity to processing establishment is high risk. If there is an outbreak the farms try to sell non clinically diseased but infected fish to be slaughtered and they have a high release of virus.
The disease and health status of the animal supplier is also very important. The risk on an individual farm will increase if the farm receives fish and/or eggs from several suppliers.
People visiting several farms (Like veterinarians, traders and governmental people) may not take care of the status of the farms posing a risk to the farms.
Put and take lakes possess a high risk and are probably more important than the fish farms in an epidemiological context but it is uncertain whether there is a legal right to control these farms.

Comments:
B. B. Jensen: *It sounds like there is a problem with the enforcement of the regulation, if the farms start selling fish if they know the disease is there.*
G. Bovo: *Before the veterinary service is informed of a clinical outbreak the farmers sell the fish that does not show disease yet, they sell these fish cheap to earn at least some money on them. This can spread the disease.*

O. Haenen: *In the Netherlands we also have a lot of small ponds, which we cannot survey.*
G. Bovo: *The problem with the put and take lakes is that they import from everywhere.*
R. Rahkonen: *We have small ponds that get stocked in spring and emptied in the fall; we solve the problem by controlling the suppliers.*

Passive surveillance:
(Reported by Marios Georgiadis)
The group believes that passive surveillance is extremely important and has been overlooked in the past where the consensus has been that regular inspections are the solution to disease control. The group thinks it is the other way round. We have to take advantage of the fact that there are so many people coming into contact with the fish to identify upcoming disease problems. There are three steps to this: 1) People recognising they see something irregular – education, 2) The reporting that they have seen something 3) And what is done with the information received from these reports.
The consequences of reporting disease outbreaks have been very serious for some farms that have lost a lot of money reporting. If people are not met in the right way (taken seriously) they will not report again, so there should be a set of obligations for the receiver of the information.
The willingness may be very different in the different countries and what works in one country may not work in another.
Punishment might solve a few problems but definitely not all. Compensation will definitely give the farmer a bigger incentive to report. Farmers associations' codes of conduct may help to raise the willingness to report.

Comments:
O. Haenen: *If I understand you right a big part of passive surveillance is active.*
H. Hellberg: *The biggest incentive for reporting is the development of a cure.*
G. Bovo: *When I started to work with fish diseases, we had 100 % infected farms. When we adopted the directive we suddenly had no infected farms. When the competent authority goes to collect the fish, they call to make an appointment, and when they come the farm is cleaned and no dead fish can be found.*
N. J. Olesen: *You said that regular inspections are not the solution?*
M. Georgiadis: *No, I said it is not a solution on its own. It should be on top of the passive solution.*
N. J. Olesen: *When the farmers cannot live with a disease, like VHS in Denmark, they will take part in a surveillance scheme.*
B. Hill: *Passive surveillance is not passive on behalf of the farmers. For them it is active surveillance and should be.*

Concluding remarks:
B. Hill: W*e are still a long way from designing the risk-based surveillance.*
B. B. Jensen: *The commission need comments from all of you concerning the risk-based surveillance for the drafts to be prepared.*

11th Annual Meeting of the National Reference Laboratories for Fish Diseases, 6-7 June 2007

Dr. Niels Jørgen Olesen welcomed all participants to the 11th annual meeting. Each year we say we cannot grow bigger but we managed once more. This year, there were 60 participants from 35 countries. A special greeting was given to the countries that are here for the first time, namely Serbia, Kosovo, Republic of Macedonia and Bosnia & Herzegovina (the later after a long absence). TAIEX was acknowledged for helping non-EU countries to participate.
Greetings were conveyed from Ellen Ariel, who has been the coordinator for the CRL for the last ten years, but has now transferred to research.
An introduction to the scientific programme was given. The first two sessions had been held as a workshop already, so the sessions were continued from there. The first session was an update on important fish disease in Europe and their control, based on problems reported in the different member states, and starting with an overview presented by the CRL.
On Thursday, the future of the CRL will be discussed.
Some practical issues were addressed, and then the director of the institute, Kristian Møller, gave a presentation on the new institute.
The institute used to be part of Danish food and Veterinary research (DFVF), which has now merged with the Technical University of Denmark (DTU) and become two separate institutes, of which we are part of the National Veterinary Institute (VET). VET employs 320 researchers and technicians and consists of 3 departments placed in Copenhagen, Aarhus and on the island Lindholm. The institute activities are public sector consultancy, research, education and innovation. Since it is now part of a university, the education part will be much bigger in the future.
The institute covers animal health diagnostic services, surveillance and control and research for contagious diseases and is therefore part of the contingency plans.
The director welcomed everybody once again to Copenhagen.

SESSION III: UPDATE ON IMPORTANT FISH DISEASES IN EUROPE AND THEIR CONTROL

Chair: Rob Raynard

Trends in Aquaculture production in Europe

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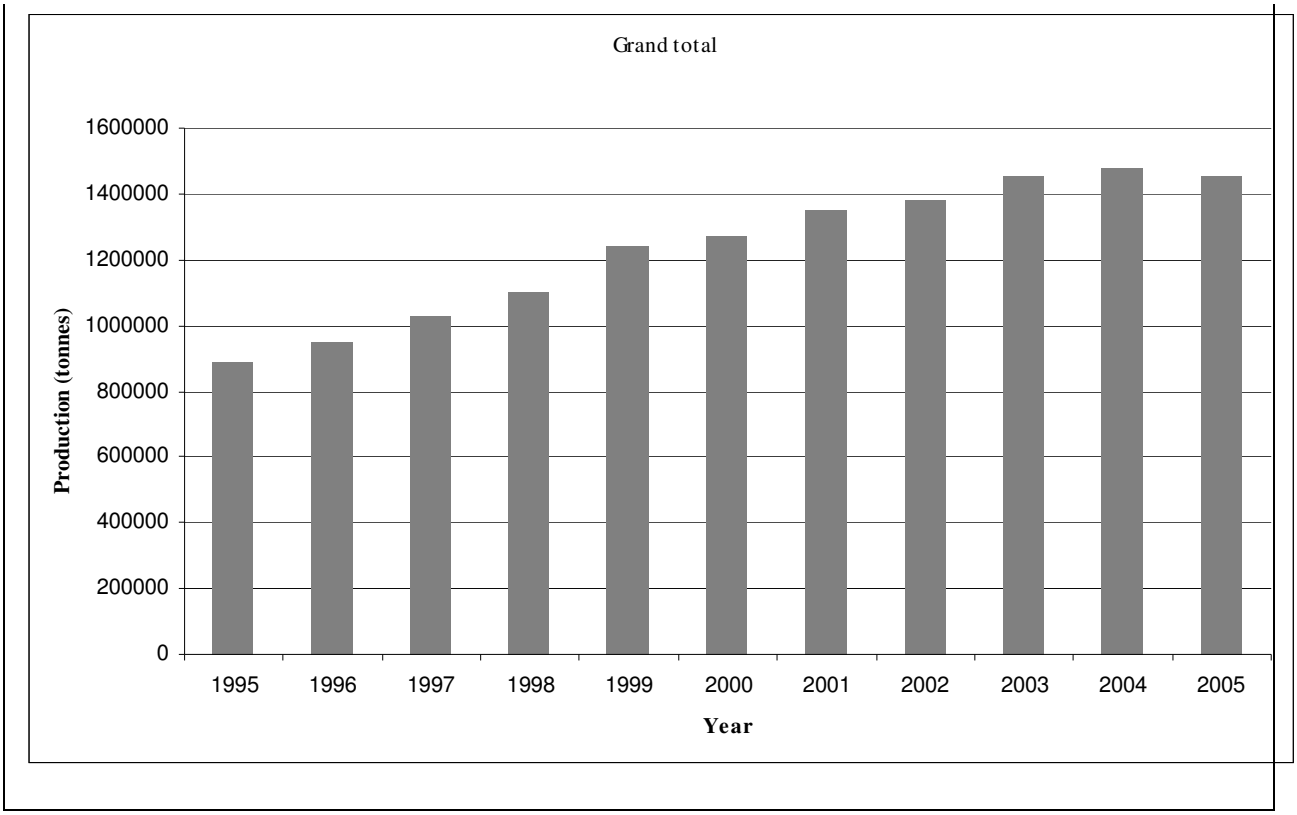
Abstract: At the annual meeting for fish disease laboratories in 2006 it was agreed that the production overview should be extracted from the FIGIS database in the future. These production data are obtained by Customized national questionnaires on aquaculture (FISHSTAT) sent annually by FAO. There is a time lag in the data, so the most recent data will be from 2005.
For all EU-countries, the relevant data has been extracted and can be found in the booklet distributed at the meeting. This presentation gives an overview of the development in production in the years 1995-2005, for both EU member states and surrounding countries that are included in the FIGIS database. Special attention is 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 as shown in the table below.



Notes from the presentation:
There has been an increase in the grand total of production for the last 10 years in Europe. It seems that we have reached a steady state in the last four years, but we do not have the numbers for 2006 due to a lag in reporting.
The production data can give an overview of susceptible species, and whether new species are being introduced. From the Survey&Diagnosis questionnaire it seems that there are many more carp farms than rainbow trout farms, but this is probably artificial since many have reported the number of ponds and not farms for carp.
It was demonstrated how to search the FIGIS database where the data originates.
The data in FIGIS is obtained from FAO (FISHSTAT). If they cannot find any data they estimate and note the number with an F. During the last ten years, the countries that have production in inland waters (freshwater) seems quite steady for the smallest and the biggest producers, whereas it seems that the middle producers are increasing their production. For the marine producers, especially Ukraine and Serbia has increased their production. Among the bigger producers UK and Norway follows each other in rise of production, though the scales are different.
It is also possible to look into production of new species, such as tilapia, which is now produced in the Netherlands and Belgium; UK stopped their production ten years ago.
The production of eels does not seem influenced by its threatened status as of yet.
In conclusion, FIGIS is easy and fast for individual countries, data can be found for an extended time period, the data are ”official” but the lag in data is probably the biggest annoyance.

Comments:
B. Hjeltnes: *You showed us a steady production. I know the production of Atlantic salmon has gone up, so what species has gone down?*
B. B. Jensen: *I do not know.*
G. Bovo: *In Italy there is a decline in production of rainbow trout, as people now eat seabass and seabream, so that production has gone up.*

R. Raynard: *In Scotland there are two ways of calculating the production: whole fish and gutted fish. If countries change the way of reporting from whole to gutted that may be part of the explanation.*
K. Denham: *They ask for production for human consumption, but there is also a big production for restocking.*
B. B. Jensen: *The FIGIS data are the official data, we have earlier discussed the quality of the data that you provided us, and it is really complex.*
O. Haenen: *Did you also look into the data from FEAP as they may be more recent?*
B. B. Jensen: *No, we only used FIGIS.*
B. Hjeltnes: *There may be a tendency that in the freshwater there is a decline, but in marine waters there is and will be a rise. Also, you may not get all the production data as the market may be reluctant to give the right production figures.*

Overview of the disease situation in Europe

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Abstract: 1) Status and emergence of VHS and IHN in Europe
2) Other fish disease monitoring programmes in EU

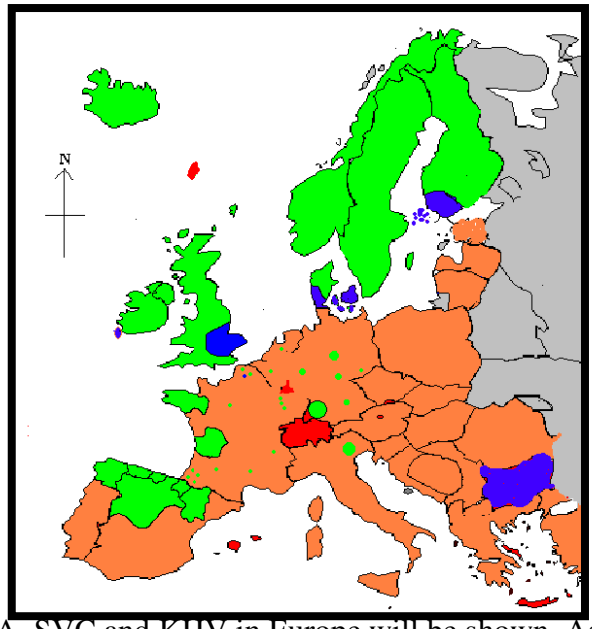
Data on survey and diagnosis on fish diseases in Europe in 2006 were collected again this year and compared to previous years some changes were again made in the questionnaire. A reason for this is the implementation of the New Council Directive 2006/88/EF Annex IV part B where ISA, SVC and KHV was added 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. The only non-approved farms in the Northern Atlantic are situated at the Faeroe Islands. Most of the "old" Member States have a number of farms approved in non-approved zones.

Green: VHS- & IHN free.

Blue: IHN free.

Red: Not approved- free zone.



For the first time the occurrence of ISA, SVC and KHV in Europe will be shown. As in previous years, however, the de-facto spreading and significance of the non-exotic diseases cannot be retrieved from the S&D questionnaire.

Notes from the presentation:
This year we received answers from most of you and we are very grateful for that. If you have corrections, please contact us (nni@vet.dtu.dk). The last data came in last week, so we have only had very little time to look at them.
You can find a summary for each country in the booklet.
We asked you all to produce a map showing were the outbreaks have occurred, unfortunately only a few have given such a map
Much of Europe does not have zone status for VHS/IHN. England reported their very first outbreak of VHS in farmed rainbow trout, in Denmark we have experienced very severe outbreaks with high mortality, whereas in Finland VHS seems to have temporarily disappeared without being eradicated.
There are many farms of unknown status in France and Germany. In the next few years, all farms in EU has to be registered according to the new Directive 2006/88, which will give more reliable data in the S&D. (However most of these farms will fall into Category III – Undetermined, and will thus still have disease status "unknown")
Regarding IHN, no significant changes have been recorded.
ISA has now changed from being an exotic List 1 disease to be a list 2 "non-exotic" disease in CD 2006/88. Nevertheless no report of ISA in EU were reported. No outbreaks were reported from the Faeroe Islands last year either, but the HPR-0 "wild type" was found by PCR at 2 occasions.
SVC has changed status from list 3 to list 2, and we have now to think about how to survey for this disease and how to apply zone status. The few reports of outbreaks of this disease come mainly from the middle of Europe.
A lot of isolations of KHV have been made in 2006, especially in Germany.
BKD programmes are now in place in Ireland, UK, Norway, Finland, Iceland, Denmark Belgium and Slovenia. Many countries also survey for IPNV.
Other findings included VNN and *Lactococcus garvieae* in Greece and Italy, *Herpesvirus Anguilla* (HVA) in Denmark, Germany and the Netherlands, Sleeping Disease (SD) in Spain and France, and Pancreas Disease (PD) in Norway.
In conclusion there were few changes from 2005 to 2006 and more countries reported on the diseases newly introduced in Council Directive 2006/88.

Comments:
R. Raynard: *Any idea why VHS outbreaks has become more severe in Denmark?*
N. J. Olesen: *Maybe the fish has become more naïve, making them more susceptible. Another explanation may be the production has become more intensive. There has also been a shift from less pathogenic to more pathogenic strains. When we do infection trials with older Danish isolates and Finnish isolates the pathogenicity is much lower.*
B. Hjeltnes: *Any idea about the economic costs of these diseases?*
N. J. Olesen: *It is difficult for us to assess this but it is very interesting questions, and it would be nice if someone would look into this.*
O. Haenen: *In the questionnaire you ask about farm cases, but what about the wild fish?*
N. J. Olesen: *I would expect people to report of wild fish under the point with exceptional events. Are there any other remarks about other things we should change in the S&D questionnaire?*
O. Haenen: *Ask people to add references it they have any references regarding outbreaks and other things asked for in the S&D.*

VHS-Outbreaks in Switzerland in 2006

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Abstract: In 2006 two series of VHS outbreaks were recorded in Switzerland.
A first case was diagnosed in a farm (A), which had been sold to a new owner 1 month before the outbreak. After the virus had been found in one single tank a fast spread within the farm was evident. This led to the immediate stamping out of the whole stock. The farm had been tested for VHS, IHN and IPN several times in previous years with negative results. The origin of the virus was not clear. There is only a very small brook without fish leading to this farm. One possibility might be eviscerated trout imported from Italy. These fish are processed within the localities of the farm. Virology of these fish showed to be negative however it has to be considered that virology was done on rather minor parts of kidney which could still be found in the carcasses. A week before the outbreak was recorded fish had been sold to a restaurant which kept the trout in a cage fixed in a river. After virus diagnosis in the farm the fish from the cage were immediately removed and analysed for the presence of VHS. Both, symptoms and virus, respectively were found. Several hundred meters below the cage in the river a large trout farm (B) is situated which is run with river water. Analyses of a first sample from this farm resulted in the demonstration of IPN but not VHS. However, 1 month later in some tanks of this farm B fish showed typical symptoms for VHS and the presence of the virus could be demonstrated. The trout of this farm are routinely transported to farm (C) in another canton for slaughtering. In this farm C an outbreak of VHS was also recorded. Fish sampled from further farms in the vicinity of farms A and B as well as wild fish from several nearby brooks and rivers were all negative for VHSV. Sequencing of the full length G-gene showed high identity of all isolates. All of them belong to Genogroup 1a. They cluster together with a recently described Romanian isolate as well as with a number of Italian isolates.

The first case of the second series was found in a very small hobby farm (D). Rainbow trout with clinical symptoms showed to be positive for VHSV. Fish from a nearby farm (E) had been added to the existing stock shortly before the outbreak of the disease. The owner of this nearby farm (E) had sent in fish for analyses due to elevated mortality. This mortality could be attributed to bacterial gill disease while no symptoms revealing a viral disease were found. The virological examination was negative. However, fish taken from this farm E by the fisheries authorities one week later showed VHS-symptoms and were positive for VHSV. This was also true for fish brought to the farm from a hobby pond in which problems had been experienced (F). These problems had been attributed to adverse environmental conditions due to a landslide. The owner of the farm (E) regularly bought fish from three farms in the canton of Jura and sold fish to hobby farmers in the vicinity. While all three farms in the Jura, where fish were bought from, were negative for VHSV, the virus could be demonstrated in a further hobby pond (G) where trout from farm E had been delivered. Stocks from all affected facilities were stamped out. Sequencing of the G-gene showed that all isolates belonged to Genogroup 1a and that they all cluster together. Variation was found in one to six nucleotides. The viruses from this second series vary from those of the first series in 5 nucleotides. It is probable that the source of all cases of series two was farm E but how the virus came into this farm is not clear.

Notes from the presentation:
The first series of VHS-outbreaks occurred in farms of two cantons in different river basins in the Rhein and the Rhone. The conclusions on the investigation of this outbreak was that the first farm had sold fish to farm b, and farm c was probably infected by downstream water, and there had been transfer of virus with fish from farm C to farm D in lake Geneva. The origin of the virus in farm A is unknown, possibly eviscerated fish from Italy processed on the farm, although virus has not been found in the eviscerated fish.

From the investigation of the two series of outbreaks, it was concluded that there is evidence for virus transfer via transport of infected fish and water, and that it was different virus isolates in the two series.

Comments:
G. Bovo: *Which wild fish did you check?*
T. Wahli: *Brown trout and only a limited number were checked. No rainbow trout were caught.*
G. Bovo: *Brown trout are less susceptible to VHSV.*
T. Wahli: *The temperature was approximately 11°C.*
R. Raynard: *Does this tell us anything about how virus is changed when transmitted?*
D. Stone: *There were very few substitutions in the virus; do you think it was random substitutions?*
S. Madsen: *I think it was random substitutions.*
D. Stone: *I guess in the early stages you may see a few changes as the virus load at infections has been low.*
N.J. Olesen: *The whole G-gene has been sequenced and only 1/1500 has changed. It is quite seldom we have 100% sequence match during a VHS outbreak in several farms, only when we take isolates from the same outbreak in a farm and from the same pond, 100% match is observed.*

Experiences with VHSV diagnosis in Austria 2006

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Abstract: In 2006 VHSV has been detected in rainbow trout from 7 facilities. With one exception all infected facilities are small and rear trout only for everyday consumption. One farmer selling fingerlings was suspected to be responsible for distributing the virus unintentionally within these small units. This suspicion was not confirmed.
Regarding VHSV epidemiology no new developments were noticed. Based on glycoprotein gene sequences we carried out a molecular phylogenetic study on a panel of VHSV samples isolated during the last decade in Austria, and compared them to sequences from other countries. The Austrian VHSVs proved to be genetically diverse and displayed all types of European strains. The aquacultural landscape in Austria with many small facilities but lack of an approved VHSV free zone may be the reason for such a diversity of VHSV strains.
A trend for improved epidemiological care and an increase in the number of approved single bigger farms, however, is still ongoing with certain setbacks, as it happened at the beginning of this year when one of four ovarian fluid samples originating from a rainbow trout farm, which was close to EU approvement, tested VHSV positive. The virus was isolated in BF-2 cells and identified by ELISA, as well as by RT-PCR and IFT, and the result was confirmed in the Community reference laboratory.
Concerning laboratory diagnostics we wish to address two issues: i) in different well established commercially available ELISA kits used for antigen detection, some field samples are not only VHSV positive but also react with polyclonal antibodies against rhabdovirus of carp, SVCV; and ii) in cases of fish of minor size without clear pathology the separate examination of brain tissue is advisable.

Notes from the presentation:
The big majority of small rainbow trout facilities avoid VHSV surveillance. Thus in 2006 there were more cases of VHS outbreaks to detect than in 2005.

Comparative examinations of VHSV samples isolated previously revealed the presence of at least 2 subtypes of European VHSV. The isolates clustered into 4 branches, one consisting of neutralizable serotype 1 isolates, another one of non-neutralizable serotype 3 isolates.
The severity of the outbreaks seems to have been declining in the recent years.
When diagnosing the disease in one case, organs have been tested separately according to fish size and clinical signs. When tested directly only the brain of small fish without symptoms was clearly positive in ELISA as well as in PCR, whereas the brains of bigger fish showing symptoms of disease were negative in ELISA, their hematopoietic organs were not only VHSV- but also SVCV positive

Comments:
S. Zrncic: *We experience that organs test negative in ELISA, but positive after propagation in cell lines.*
O. Schachner: *We experienced the same.*
N.J. Olesen: *How do you explain the positive SVC result in the test-line kit?*
O. Schachner: *Probably an unspecific reaction with fish components occurred. The cell culture passage was no more positive.*

Update on the current status of VHS and IHN in Spain

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Abstract: Spain is one of the European countries with a greater aquaculture production. In the last six years there has been a downturn of freshwater-farmed fish meanwhile the production of marine fish species is steadily increasing.

The main species of freshwater-farmed fish in Spain are: Rainbow trout *(Onchorynchus mikys),* Sea trout *(Salmo trutta),* Tench *(Tinca tinca)* and Sturgeon *(Acipenser baerii).* The most important marine species farmed fish are Seabream/Seabass (*Dicentrarchus labrax / Sparus aurata)* whose production has doubled in 6 years, from 12000 tonnes in 2001 to 30000 in 2006. Another species which has a significant production is Turbot *(Psetta maxima)* that has progressive increased, reaching 6300 tonnes in 2006. It is important to point out the fattening of tuna (*Thunnus thunnus*) with 3000 tonnes in 2006. Apart from these there are other emerging cultivations of aquatic fish as Common sole (*Solea senegalensis),* Meagre *(Argyrosomus regius), etc…*

In relation with the National surveillance program and diagnosis of VHS &IHN carried out in Spain in 2006 there are 203 farms with susceptible species. 172 are situated in a VHS & IHN free zone and 3 are approved of free status in a non-approved zone, furthermore there are 6 farms that are submitted to sampling to obtain approved of free status. All this farms are considered to be free from these diseases according to national surveillance. On the other hand, there are 22 farms with susceptible species for which the infection status of VHS/IHN is unknown.

Notes from the presentation:
There has been a decrease of 25% in the production of freshwater fish species since 2001, and half of the total production is now mariculture.
Turbot is produced in the North of Spain and tuna in the East and South. Seabream and seabass are also produced in the East, South and on the Canary Islands.
Most of the northern part of Spain is VHS/IHN free zone, and in the South, six farms are sampled to obtain free status while three are already declared free.

The National Reference Laboratory is placed in Madrid, and in the North there are five designated laboratories.
The first IHNV-strain was isolated in 2005 from a private farm undergoing a sampling programme to obtain free status. The affected species was rainbow trout and 15 pools from 150 fish were analysed. The diagnosis was done using cell culture isolation, neutralization, IFAT and PCR. An isolate was sent to the CRL, were it was sequenced and found to belong to genogroup M.
Further examinations were negative. In April 2006 all the fish were slaughtered and the farm now produces sturgeon.

Comments:
G. Bovo: *Did the farm produce their own juveniles?*
M. Vigo: *We do not know, but the owner has two more farms, declared free, in the same area which rear juveniles.*
Q: *Do you have any information on tuna health status?*
P. Fernandez: *In 2006 there has been a surveillance research program for VHS and nodavirus focused on the fish used to feed tuna.*
K. Denham: *Has VHSV been found historically in Spain?*
M. Vigo: *There has been a publication about VHS detected by RT-PCR in wild salmon in Galicia. The national veterinary services have asked to the authors in which basins the virus was detected in order to know if there are farms in those zones to control but at the moment they have not obtained answer.*

Fish health trends and developments in Norwegian aquaculture 2006

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Abstract: The total production of farmed Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*) amounted to more than 650 000 tonnes in 2006. The production of Atlantic cod (*Gadus morhua* L.) was 9500 tonnes, compared to 5500 in 2005. The Norwegian fish farming industry is moving towards fewer and larger companies, many of them multinational corporations. Most of these companies have an integrated production covering all or several steps in the production-cycle from egg to table.

The disease situation for farmed Atlantic salmon in 2006 resembles that of earlier years, with pancreas disease (PD), heart and skeletal muscle inflammation (HSMI) and infectious pancreatic necrosis (IPN) being the main problems (Table 1). ISA was not a major problem in 2006, with the lowest number of outbreaks since 1994 and 1995. IPN caused large losses despite extensive vaccination. HSMI is now recorded along the entire coast. More seriously, PD continues to spread, in 2006 the first cases were recorded in Møre and Romsdal. Cardiomyopathy syndrome (CMS), proliferative gill disease (PGI) and winter ulcer disease continue to cause losses.

Table 1. Number of sites diagnosed

	2003	2004	2005	2006
ISA	8	16	11	4
IPN	178	172	208	207
PD	22	43	45	58
HSMI		54	83	94
Piscirickettsia	5	0	0	1
Furunculosis	2	3	1	3
BKD	1	1	2	0

The most significant finding in marine fish in 2006 was the diagnosis of viral nervous necrosis (VNN) in cod. VNN has not previously been reported in cod in Norway. The three VNN outbreaks in cod occurred in fish from 5g to 1.5kg. Francisellosis, caused by *Francisella philomiragia* subsp. *noatunensis* (Mikalsen *et al.* in press), was diagnosed at 6 sites from Rogaland in the south to Nordland in the north. Vibriosis is the most common problem in cod farming, and *Vibrio* (*Listonella*) *anguillarum.* serotype O2 is usually isolated. However, a new variant of *V. anguillarum* serotype O2 has been identified. The variant shows positive agglutination with anti- O2 rabbit sera, but differs from "normal" O2 in antigenic, phenotypical and genetic criteria. In halibut (*Hippoglossus hippoglossus* L.), two outbreaks of VNN and one of IPN were registered.

Notes from the presentation:

A report regarding ISA in Norway 2003-5 has been issued and is available at the website http://www.vetinst.no/

For IPN there have been no major changes in the disease situation from 2005. Approximately 45% of the cases occur in the hatchery phase, mainly in fry, and rainbow trout is almost exclusively affected during the hatchery phase. The most economically important problem occurs in smolt 3-4 months after transfer to sea.

Heart and skeletal muscle inflammation (HSMI) has only been observed in Atlantic salmon and only sea water sites have been affected. It can occur year round and mid-Norway remains the most frequently affected area. The number of cases is increasing, partly due to better detection but there is also a true increase.

Pancreas disease (PD) affects Atlantic salmon and rainbow trout and the disease occur in on-growing sea cage sites, where on average the outbreaks occurs 8 months post transfer to seawater. It is a very costly disease due to loss of growth and downgrading of fillets. PD is not notifiable, but the competent authorities are considering a change of status. In addition, the disease is considered as such a problem by the fish farming industry that they have asked for it to become notifiable.

The symptoms seen in the outbreaks of Viral Nervous Necrosis (VNN) was anorexia and spiral swimming, concurrent with high water temperature, but a low mortality that decreased when the temperatures dropped. Still, the cumulative mortality was quite high in some cases.

Francisella philomiragia subsp. noatunensis has been diagnosed at 6 sites, and is probably underreported. The agent is present in wild fish, but the disease has not been observed along the Norwegian coast.

Annual reports on the fish health situation and surveillance programs in Norway are available at the website.

KHV in Germany

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Notes from the presentation:
Event though KHV is the largest virus within the family *herpesviridae*, there is now access to the complete genome of three isolates published by the Japanese colleagues under the leading of Prof. Aoki.
When using Gilad´s primers followed by nested PCR, one should beware of the risk of contamination. Since the glycoprotein gene is a reverse gene, you have to be careful when designing primers. The major envelope protein gene of KHV is flanked by membrane protein and another gene. Often PCR using structure genes are more sensitive as such at recognizing viral enzymes, especially in herpesviruses (e.g. TK, DNA polymerase)
Validation of the KHV PCR is necessary, both nationally and internationally.

The following is known about the host range of KHV:
The disease affects only common carp and koi (C*yprinus carpio*) and hybrids, but other fish such as goldfish, crucian carp, grass carp, sturgeon, sheatfish, bighead carp, silver carp, tench and vimba can be carriers. Serum or plasma, leukocyte separation and filter paper assay (FTA®) can be used for non-lethal testing by ELISA (antibodies) or by different PCR and / or real time assays.

In conclusion KHV seems to be a lymphotropic herpesvirus, and for detection several methods are available (PCR, real time PCR, IHC, IFAT, IEM and ISH). KHV latency is provable and transmission of KHV is possible from and to other fish. Best organs for detection, also in the case of latent infections, are the kidney, the gills, blood and leukocytes and then the other organs filled with blood.

Comments:
O. Haenen: *Do you have any idea of the titre?*
S. Bergmann: *Only from the isolation of KHV from carp, where the titre was 10^2-10^3 $TCID_{50}$ /ml as highest.*

Occurrence of a new subtype of North American viral hemorrhagic septicaemia virus (VHSV) in the Great Lakes

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Presented at this Annual Meeting by Helle Frank Skall on behalf of the authors.

Abstract: VHSV is an aquatic rhabdovirus that infects fish species in Europe, Asia, and North America. VHSV genotyping has revealed four main genogroups (I, II, III, & IV) which tend to correlate with geographic origin of virus isolation. Historically, North American VHSV (genogroup IV) has only been isolated in marine fish populations in the North Pacific and North Atlantic oceans; however, in the spring of 2005 VHSV was isolated from a mass mortality event of freshwater drum (*Aplodinotus grunniens*) in Lake Ontario. Subsequently this virus has been detected in fifteen different freshwater fish species and has spread throughout the Great Lakes region to include Lake Huron, Lake St. Claire, Lake Erie and the St. Lawrence River. To gain insight into the epidemiology of this emerging pathogen we performed genetic characterizations on multiple isolates taken from sites located throughout the Great Lakes area. Phylogenetic analyses based on nucleotide sequence of the nucleocapsid protein and a region of the

glycoprotein gene revealed that VHSV from the Great Lakes region was genetically related, but not identical, to isolates from the Atlantic coasts of North America suggesting that the virus was introducted into the Great Lakes via an eastern North American marine reservoir. This new North American subtype composed of Great Lake and Atlantic coast isolates is denoted as IVb as compared with isolates from the western North America that are genogroup IVa. Additionally, Great Lake isolates were characterized based on their ability to replicate in various cell lines as well as compared with genogroup IVa virus for their stability in seawater and freshwater at 4, 10, 15 and 20 degrees Celsius.

Notes from the presentation:
The history of VHS is as follows:
1931: First report in Europe of a disease similar to VHS
1962-63: First isolation of virus in cell culture
1988: First detection of VHSV in North America
1990→: Isolations from marine fish species in Asia, Europe and North America
2005: First detection of VHSV in American freshwater environment (Great lakes)

Genotypes I, II and III are European, IV is American, and the division may have happened 500 years ago. Now, it has been found in more than 45 fish species in North America.
The mid G-gene region was used for typing 64 isolates from 36 locations spanning 20 years, revealed 28 different sequence types.
The isolates from the outbreaks in the Great Lakes group together with east coast isolates in a new subgroup IVb, in contrast to west coast isolates which belong to subgroup IVa. Even though the virus was first detected from the freshwater environment from diseased fish in 2005, a re-examination of a Muskellunge found in Lake St. Clair already in 2003 revealed the presence of VHSV.
The growth characteristics are similar from the east- and west coast isolates, they grow in EPC, FHM and BF-2 cell lines, but not in CHSE and RTG (where European strains grow).
Together with two marine strains, the great lake strain was tested for viral stability in different water sources and at different temperatures.
The virus titer stayed higher in freshwater for a long period of time compared to seawater, but there was no difference between the marine and the freshwater strains.
OIE was informed of the outbreak in summer 2005, and USDA-APHIS ordered a ban on movement of fish in all states bordering the great lakes (in both Canada and USA). USA and Canada has agreed on a bilateral surveillance program to document the spatial extent of VHSV positive populations. Watersheds, zones and compartments are characterized as free, positive and suspect.
There is still no knowledge on how VHSV entered the great lakes, but since it groups with eastern North American strains, it might have come from the marine environment, i.e. with migratory fish or ballast water.
VHS has now been found in other inland waters and not just the Great lakes. VHS has spread a lot last year, into USA and UK, and the list of susceptible species is increasing.

Comments:
U. Rikula: *What sample was taken in 2003 from the great lakes?*
H. F. Skall: *Organ material from fish.*
D. Stone: *Are the Americans treating the isolation of IVa differently that from before?*
H. F. Skall: *They thought that the American genotype IV was just in the marine environment, but now they have found the virus in the freshwater environment (genotype IVb) they will try to contain/eradicate this genotype. Due to this the focus on IVa, found only in connection with the marine environment, is limited at the moment.*
G. Bovo: *Did all the new species show mortality?*
H. F. Skall: *I do not know.*

Major disease problems in the Mediterranean aquaculture

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Abstract: The Mediterranean aquaculture is a rapidly growing industry reaching, during 2006, approximately 210,000 tons with seabass and seabream representing 91%. Other species like sole (*Solea senegalensis*), meagre (*Argyrosomus regius*), turbot (*Psetta maxima*) and northern blue fin tuna (*Thunnus thynnus*) are very promising candidates for the immediate future.
Old and new diseases play an important limiting role both in juveniles and on-growing facilities. Parasites like *Amyloodinium ocellatum* and *Cryptocaryon irritans* still represent a serious threat, causing significant losses if not properly treated, particularly when high temperatures occur and water renewal is limited.
Ceratothoa oestroides is a macroscopic isopod affecting gills and oral cavity particularly in juveniles in which feeding is hampered. Among myxosporidians, which have normally a low economic impact, *Enteromyxum leei* may cause severe mortalities in juveniles or sub-adult fish. Sea bream may suffer losses up to 15% and no treatments are unfortunately available. Pasteurellosis (*Photobacterium damsela subspecies piscicida*), Vibriosis (*Vibrio anguillarum; V. algynoliticus* and *V. harveyi*) and Myxobacteriosis due to *Tenacibaculum maritimus* represent the most important bacterial diseases commonly found in many geographical regions. Vibriosis is easily controlled by prevention. In fact consolidated commercial vaccines providing a robust protection are available. Pasteurellosis is a serious problem particularly in seabream hatcheries where the infection may appear during larval stages when no vaccination may be effectively occurred adopted. During early Summer 2006 serious losses affecting mainly wild mullets (*Mugil spp.*) along several Mediterranean coasts. Tons of adult fish, showing typical chronic lesions, died and were found on the beach. The social impact was very impressive particularly in touristy areas. Abnormal high water temperatures and the presence of high biomass of susceptible farmed population are suspected to play a significant contribute to the epidemic observed.
Among viral diseases Encephalopathy and retinopathy (VER) represents the only significant threat for seabass industry. Because of the large host range other species may be affected, like shi drum (*Ombrina cirrosa*). During the recent years losses reaching 100% mortality have been detected in a few seabream Mediterranean hatcheries. In one farm situated in south Italy no production has been obtained during 2006 because of the appearance of VER and the farmer decided to avoid any seabream production during 2007 and discharge all the brood stock.
According to a 2006 questionnaire submitted to several people working in the field the control of the disease is carried out in some important hatcheries through brood fish testing by PCR and ELISA and the application of strict hygiene measures.
The existence of a large number of susceptible species makes the control of the disease very difficult and underlines the urgent need for an efficient vaccine and establishment of official control measures. In the meanwhile it is crucial to adopt strict hygiene procedures, avoid the introduction of infected brood stock and maintain a continuous monitoring at farm and regional level.

Notes from the presentation:
Apart from the disease problems mentioned in the abstract, lymphocystis virus (iridovirus) can affect more than 100 species in both marine and fresh water. It affects mainly juvenile fish, and the main external lesions are cutaneous grey nodules. These signs last 1-2 months with low direct mortality, but causing reduction of the production circle.
Viral encephalopathy and retinopathy has a large host range, but seabass is the target host in the Mediterranean area. All ages are affected, and it occurs mainly during summer or at temperatures above 22°C. The fish show erratic swimming, but no external lesions even though it causes significant losses. It has been isolated in a few wild species.

During May-June 2006 a serious epizootic affecting mullets appeared along many Mediterranean coasts. The mortality was apparently due to a heavy *Photobacterium damsela subs. piscicida* infection in correspondence with a high temperature period. High temperatures are suspected to play an important role, and the mean annual temperature has risen in the last decades. Furthermore farmed fish are suspected to maintain high bacterial pressure in the environment.
Tuna production is a promising new industry. Unfortunately no official health control has been yet established for tuna and we hope the new directive could give us a legal tool to start control of these farms. Tuna are currently fed only with frozen whole fish originating from all over the world which could be very dangerous not only for tuna but for the whole fish population leaving in the Mediterranean. We know in fact that fish wild population may harbour serious pathogens which may spread in different host in different geographical areas.

SESSION IV: TECHNICAL ISSUES RELATED TO DIAGNOSIS

Chair: Sven Bergmann

Validation of serological methods used for diagnosis of VHS and IHN

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Abstract: In warm-blooded animals, serological methods are considered as reliable diagnostic tools and used in routine for successful eradication policies in many countries. Even though antibodies against VHSV have been evidenced for the first time in trout 36 years ago, and antibodies against various fish viruses (IHNV, SVC, IPN, ISA, SPDV/SDV, nodavirus,…) have frequently been reported, it is still considered that the detection of antibodies to fish viruses can not be accepted as a routine diagnostic method to be used to assess the viral status of fish farms. One of the objectives of the work package 6.1 in the EPIZONE network is the validation of serological methods for detection of VHSV and IHNV antibodies in order to establish guidelines for an international proficiency test in fish serology.
Afssa and VET-DTU have started validation of a seroneutralisation test and ELISA according to the protocol described in the "Manual of Diagnostic Tests for Aquatic Animals" from the OIE.

The first part of the validation procedure is on-going and is performed on sera of rainbow trout experimentally infected with IHNV.
In the seroneutralisation test validation, the following parameters are studied using 10 selected anti-IHNV sera:

- Cell lines and cell concentration
- Threshold sensitivity
- Virus concentration
- Virus strain
- Complement concentration
- Time of reaction
- Repeatability
- Specificity
- Serum or plasma
- Temperature of conservation

After the different parameters have been controlled using sera from experimental fish, the test will be applied to sera from farmed trout infected with IHNV. A similar protocol will be applied to antibodies against VHSV.

The last stage of validation will consist of a comparison of results obtained by different participants of an interlaboratory proficiency test. The maintenance of validation will depend on reproducibility of the test between laboratories.

Notes from the presentation:
The gold standard for identification of VHS and IHN is cell culture, but VHSV and IHNV are rarely cultivated when no clinical signs are seen or the temperature is above 15°C.
Serological methods have been in use for a while, but they need validation.
One objective of WP 6.1 of EPIZONE is serology of VHS and IHN, to be used for screening of populations.
The validation is performed according to the OIE guidelines, in which there are four steps:
1. Optimisation, repeatability, sensitivity and specificity.
2. Performance characteristics, threshold determination, Diagnostic se/sp and comparison and harmonisation of the assay
3. Establishing the reproducibility of the assay.
4. Programme implementation and monitoring of the assay performance.
For validation of reagents for the IHNV sero-neutralisation test, sera from IHN-infected, VHS positive and true control negative from SPF trout were tested together with sera from trout with anti-IPN and/or anti-SDV. The IHNV strain used was isolate N61.
The experimental conditions were as follows:
EPC and CHSE-214 cell lines were used, in four concentrations; the virus was in concentrations and the complement in three. The trays were incubated for 2hrs at 14°C or 16hrs at 5°C.
The results showed that the EPC-cell line were more suitable than the CHSE cell line, and the optimal combination was a cell concentration of 20-30x10^6 cells/96 well plate, a complement dilution of 1:30 and a time of 16hrs at 5°C. No cross-reaction when using VHSV was seen and there were no neutralisation of IHN by anti IPN, anti SDV or anti VHS.
The diagnostic sensitivity was 90.5% and the specificity 97.5%.

Comments:
D. Stone: *Do you plan to include other tests (i.e. quantitative PCR) in the ringtest so you can know what the level of infection is in the fish?*
J. Castric: *We can use PCR-test, but the advantage of the serology is that you do not have to kill the fish.*
N.J. Olesen: *The diagnostic sensitivity was very good, but it maybe even better, because the fish may not have developed antibodies. Therefore the false negatives may actually be truly negative even though they should be positive because they were infected.*

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

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Abstract: Diagnosis of viral haemorrhagic septicaemia virus (VHSV) is based on virus isolation on fish cell lines and subsequent identification by serological methods. In the recent years diagnostic alternative procedures as conventional RT-PCR or real-time PCR on tissue samples or for virus identification has been desired.
In the present study, a RT-PCR assay for amplification of the nucleotide gene (N gene) of VHSV in cell culture was validated. For comparison, twelve different strains of VHSV representing the known four genotypes (Snow et al. 2004) were analysed by RT-PCR using 2 different primer sets (Snow et al. (2004) and Knüsel et. al (In press)), respectively. The primer specificity was also tested against a panel of non-VHSV fish viruses. The examined VHSV

isolates were propagated on both BF-2 and FHM cells in order to evaluate whether these often used cell lines influence the specificity and sensitivity of the RT-PCR assay.
The assay using the primers described by Snow et al. was obviously the most successful. This set was further assessed by testing its detection limit, reproducibility, sensitivity and specificity on VHSV propagated in cell culture, finally, an expanded panel representing 50 VHS isolates from a broad geographic area, including both fresh water and marine isolates, was analysed.
Plans for assessing the diagnostic sensitivity and specificity for detection of VHSV directly in organ material from fish will be presented.

References

Snow N., Bain, N., Taupin, V., Cunningham, C.O., King J.A., Skall H.F., Raynard R.S. (2004). Genetic population structure of marine viral haemorrhagic septicaemia virus (VHSV). *Diaeases of Aquatic Organisms* 61: 11-21

R. Knüsel, S. M. Bergmann, K. Einer-Jensen, J. Casey, H. Segner and T. Wahl[1] Virus isolation versus RT-PCR: Which method is more successful in detecting VHSV and IHNV in fish tissue sampled under field conditions? *Journal Of Fish Diseases* (In press)

Notes from the presentation:
The validation is done according to the quality assurance (QA) system DS/EN ISO/IEC 17025, 2. Version 2005-06-10.
One of the isolates used in the validation did not show up positive for VHSV. The virus isolate was inoculated onto BF-2 cells and FHM cells, and the isolate showed a cross reaction with IHNV. On FHM cells the IHN virus had out competed the VHS virus but in BF-2 cells there was a reaction with both primer sets investigated.
Primers designed by Bergmann are sensitive but there might be problems with unspecific bands, whereas the sensitivity and specificity for the primers designed by Snow seems ok.
In total 50 isolates from all over the world has been investigated for the validation using the primers designed by Snow. The detection limit for isolate L59X is 10^{-7} and repeatability is ok but the diagnostic se and sp is still to be tested on true positive and true negative field samples.
For repeatability, a dilution series was done and tested in triplicates.

Comments:
S. Bergmann: *How was the RNA concentration of the virus?*
S. Madsen: *We did titrate it, but I do not have the concentration here.*
S. Bergmann: *Did the unspecific bands fit to the size?*
S. Madsen: *The bands in the BF-2 cells were very close to VHSV.*

Classification of viral haemorrhagic septicaemia virus (VHSV) and how do we define the disease VHS?

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Abstract: All VHS virus isolates characterised so far, share antigenic epitopes that are recognised in IF and ELISA by polyclonal antibodies (PAb) raised against the VHSV reference strain Type I (DK-F1). However, VHSV can in neutralisation test be divided into 3 subtypes based on their neutralisation pattern towards a panel of 4 neutralising monoclonal antibodies (MAbs) and 1 PAb. MAbs reacting specifically with the Japanese/American isolates and not with European VHSV strains have been produced. But no MAbs exist that can discriminate between VHSV of aquaculture origin and from wild marine fish in Europe. Genetic characterisation of VHS virus has identified four major genotypes, based on sequencing of an N-gene fragment, the G-gene and the NV-gene, respectively:

Genotype I:	Several sublineages (Ia-Ie) containing European freshwater VHSV isolates (1a, 1c, 1d), Black Sea and Turkish isolates (1e) and a group of marine isolates from the Baltic Sea, Kattegat, Skagerrak, the North Sea and the English Channel (1b)
Genotype II:	A group of marine isolates from the Baltic Sea
Genotype III:	Marine isolates from the North Sea, Skagerrak and Kattegat
Genotype IV:	Two sublineages of North American and Japanese isolates, with 1b consisting of the recent isolations from the Great Lakes in 2006

However, the best resolution of genotype sub-lineages is obtained when analysing full length G-gene. Except for one MAb the genetic grouping and the serological grouping using G-protein specific antibodies do not correlate.

The marine and fresh water isolates are serologically indistinguishable, and are to some extend distinguishable by sequencing, but differ with respect to pathogenicity on the host species: Marine VHSV isolates do in general not induce disease in rainbow trout. The genetic studies have shown that the marine virus group possesses the majority of diversity, but so far, attempts on identifying a marker that may link genetic and pathogenic characteristics has been unsuccessful.
The presentation invites to discussion on whether the notifiable disease VHS should be defined as infection with specifically defined genetic groups of virus potentially pathogenic to rainbow trout rather than VHS virus as one group. There is an urgent need for clear and simple definition of the notifiable aquatic viral diseases, as VHS, SVC, and ISA. A proposal for such a definition for VHS is that "VHS is a disease caused by a rhabdovirus which reacts in ELISA or IF with the monoclonal antibody IP5B11". Further perspectives of using molecular tools for virus characterisation as supplement to serological tolls will be discussed.

Notes from the presentation:
Neutralizing MAb´s against VHS are all directed against the viral glycoprotein, and a system of serological discrimination was made in the 1980´es based on 3 different neutralisation patterns.
All the old VHS isolates were in serotype I, but in the 1980'es pattern II and III turned up. That was discovered when many of the isolates escaped neutralisation, but turned out to belong to serotype II and III.
Different non-neutralising MAb´s have been developed. IP5b11 includes all VHS isolates (tested more than 1000) and reacts against the N-protein, whereas MAb´s against the M-protein, has some degree of cross-reaction with IHNV.
It is not possible to use serology for clear discrimination between VHS isolates since all VHS virus isolates are serological very homogeneous.
The pathogenicity of the genotypes differ in infection trials, where pathogenicity is 40-90 %(100%) for genotype Ia, genotype Id is much lower (40%) and Ib is in general non-pathogenic for rainbow trout in experimental infections by immersion but has been seen to give 25% mortality (Swedish farmed rainbow trout isolates).
VHS from turbot is not pathogenic for rainbow trout, except for one old German farmed turbot isolate.
Genotype IVa is pathogenic for herring, pilchard and Atlantic salmon. Genotype IVb is pathogenic to at least 15 fresh water fish species, but not to rainbow trout by immersion experiments.
Pathogenicity and genotype do not correlate. In general pathogenicity follows the species from where the isolate was first isolated, genotype II, III and IV have not been shown to be pathogenic for rainbow trout yet.
Virus isolates from all farmed fish and isolates from free-living fish made in connection with clinical symptoms and mortalities should be notified to OIE.

If it is justified that certain genotypes are not present in certain geographical areas, import restrictions should be made possible.

Comments:
B. Hill: *You say that everything that reacts with antibody IP5B11 is VHSV, but the policy should depend on genotype? What if you have outbreak of II or III in marine farms, how should that be treated?*
N. J. Olesen: *It should be treated as a VHS-outbreak, and restriction on sales should be the same. When you have one genotype of virus, you should not be allowed to just import any other genotype of VHS-virus.*
G. Bovo: *Do you have data from experimental trials with the isolate from Turkish seabass?*
N. J. Olesen: *no, we have not been able to obtain that isolate.*

The use of VHS-IHN samples for screening of other fish pathogens

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Abstract: The National Veterinary Institute (NVI) in Norway receives yearly approximately 1300 pooled organ samples from 400 fish farms located along the coast for surveillance of VHSV and IHNV according to Directive 91/67/EEC and Decision 2001/183/EC. The samples are collected by the Norwegian Food Safety Authority. Infectious pancreatic necrosis virus (IPNV) is often isolated from these samples since it is ubiquitous in Norwegian fish farms. Recently, salmonid alphavirus (SAV) has been frequently isolated in BF-2 cells from VHS-IHN samples collected in area with a high prevalence of pancreas disease (PD). The aim of this study was to investigate the suitability of the samples submitted for surveillance of VHSV and IHNV for screening for other pathogenic virus such as SAV and infectious salmon anaemia virus (ISAV) using real-time RT-PCR.

During January and February 2007 NVI received 3 pooled tissue samples from 10 fish from 27 salmonid producing farms from a region in Western Norway with a high prevalence of PD. At arrival at the laboratory, the tissues were homogenized and aliquots of the homogenates were transferred to lysis buffer and stored at -80 ºC until analysis by real-time RT-PCR. The remaining homogenates were further processed and inoculated onto BF-2 and EPC cells for surveillance for VHSV and IHNV. Nucleic acids were isolated from samples on lysis buffer using an automatic extractor (NucliSens® easyMAGTM), and SAV was detected using primers and probe from conserved regions of the gene encoding the surface protein E1. ISAV was detected using primers and probe from gene segment 7 according to Plarre et al. (2005, Dis. Aquat. Org. 66:71-79). If positive reactions, primers and probe from segment 8 were used for verification of ISAV.

SAV was detected by real-time RT-PCR in samples from 6 farms of which 5 produce A. salmon and 1 rainbow trout. Interestingly, SAV was also detected by cell culture isolation in BF-2 cells in samples that were positive by real-time RT-PCR. SAV was in this case verified by immuno- fluorescence test.. ISAV was detected in samples from 1 farm producing A. salmon and which was negative for SAV.

As a conclusion, pooled samples collected for VHS-IHN surveillance may be suitable for screening for other fish viruses or other pathogens by PCR-based methods. However, the pooling of organs from 10 individuals in each sample will result in reduced sensitivity compared to the analysis of single fish. But for disease surveillance in regions or in fish farms with specific disease problems, the use of VHS-IHN samples may represent a low-cost system for screening for specific agents like SAV, ISAV and new emerging diseases.

Comments:
S. Bergmann: *How can you differ that it is PD growing in BF-2?*
H. Hellberg: *I will have to refer you to Birgit Dannevig for description of the characteristic CPE.*
N. J. Olesen: *The results are interesting because you can get better value for all the sampling we are doing.*
H. Hellberg: *Yes, the competent authorities would probably appreciate that.*
N. J. Olesen: *It is strange that SAV can now grow on cell culture while it was very difficult to propagate 10 years ago.*
J. Castric: *Yes, and Sleeping disease virus grows on BF-2 cells.*
G. Bovo: *We have received tissue material from rainbow trout from an area where there is sleeping disease but we have never seen CPE.*
F. Geoghegan: *What are the consequences for the farm when you see ISA?*
H. Hellberg: *Usually we go back to re-sample, but this time it was agreed with the CA not to take any action.*
B. Hjeltnes: *In this case we could not do anything because the fish farmers had submitted samples for VHS and IHN and not for just any test.*

Sampling and Diagnostic Guidelines for Infectious Salmon Anaemia (ISA)

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Abstract: Initially, Infectious Salmon Anaemia (ISA) was first diagnosed as a disease based on clinical and pathological finding. Patho-morphological evaluations are still a cornerstone in routine diagnostics. However, it is important to focus on mechanisms for pathogenesis and manifestations of such rather than just "typical" findings. Several methods are available for identification of ISA virus. These includes demonstration of the virus antigen by anti-ISA virus antibodies on tissue imprints (IFAT) and formalin-fixed paraffin–embedded tissue sections (IHC) and). IHC has a major advantage by linking the detection of virus antigen to pathological lesions. Cell culture isolation of ISA virus is performed using either SHK-1 and/or ASK-II cell lines. Virus isolation been regarded as the "gold standard" for ISA confirmation. Detection of virus genetic material by RT-PCR has become a rapid and sensitive method for confirmation of ISA virus and for screening purposes. Recently the detection of less virulent strains of ISA virus (HPR0) may pose a challenge for diagnoses of ISA.

Notes from the presentation:
The steps to follow when establishing a diagnosis are:
-Clinical observations and pathomorphological examinations
-Identification/confirmation of causative agent and
-Establishment of a causative association
The definition of a suspected case can be found in the OIE-manual, and for ISA this is consistent with clinical and/or pathological changes consistent with ISA, with or without clinical signs of disease, followed by isolation and identification of ISAV in cell culture, and evidence for the presence of ISA from two independent laboratory tests.
The infected fish may show a range of pathological changes from mild to severe, so the focus should be on the aetiology which is virus replication in the endothelial cell lining the inner walls of blood vessels and heart, therefore signs consistent with circulatory failure should raise the suspicion of ISA. If ISA is suspected, try to get samples for virology, PCR, serology as well as for full histology.
Identification is mostly based on immunological techniques. IFAT has the following advantages: inexpensive, fast (imprint) and a small room for interpretation. IHC is inexpensive,

more robust and links virus detection to pathology. For example interstitial haemorrhages in the kidney are almost pathognomonic for ISA.
RT-PCR on the other hand is rapid and highly sensitive and specific, real-time RT-PCR is used for virus confirmation and several primer sets have been used.
Serology should be further developed.
The question is whether cell culture can still be regarded as a gold standard?
Yes, if all isolated that grow in cell culture are pathogenic, but not if cell cultivation will support the growth of some non-pathogenic or very low pathogenic strains.

Comments:
D. Christiansen: *Could two different RT-PCRs with different primer sets function as two individual tests?*
B. Hjeltnes: *Theoretically yes.*
K. Falk: *PCRs are based on the same principle, whereas IFAT and IHC are based on detecting the antibody.*
B. Hjeltnes: *Normally two tests based on different technology are required*

EPIZONE: Results of global Koi Herpes Virus questionnaire

Olga Haenen[1] & Niels Olesen[2] & many colleagues (see below)
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Abstract: EPIZONE is a big EU network of excellence project within FP6 (www.epizone-eu.net), with 20 partners from Europe, and China, Turkey, FAO and DiVa. Its mission is to improve research on preparedness, prevention, detection, and control of epizootic diseases within Europe to reduce the economic and social impact of future outbreaks of emerging/notifiable diseases, like Foot-and-mouth disease through increased excellence by collaboration.
Koi Herpes Virus Disease (KHVD) is a world wide emerging disease of *Cyprinus carpio,* koi and carp. A detailed questionnaire on KHV was designed, for EPIZONE. It was sent to > 65 countries in Dec 2006 – May 2007. By the start of May 2007, 39 countries had responded, i.e. 60%.

Summary of results: KHV was detected from 1998. 18 countries reported to be KHV positive (Austria, Belgium, China, Czech Republic, Denmark, France, Germany, Hong Kong, Indonesia, Ireland, Israël, Japan, Korea, Netherlands, Poland, Switzerland, UK, and USA) , 1 suspicious (Russia), and 20 negative (Australia, Croatia, Cyprus, Estonia, Finland, Greece, Hungary, Italy, Latvia, Mexico, Norway, Philippines, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Turkey and Ukraïne). In 17 countries KHV was found in koi, in 12 in cultured carp, and in 4 in wild carp. The maximum number of outbreaks varied per year: in 2004: koi 68 (GER), cult(ered) carp: 38 (Japan), wild carp: 872 (Japan); 2005: koi 110 (GER), cult carp 30 (Japan), wild carp/koi ponds 280 (Japan); 2006: 77 (NL), cult carp 30 (Japan), wild carp/koi ponds 151 (Japan). The outbreaks were detected in small & big koi and carp. The mortalities varied: in koi 10-100%, cult carp <10%-100%, and in wild carp mortalities were often high but unknown. Clinics were like described in various KHV articles. Diagnosis: 27 used PCR, 1 nested PCR, and 4 TaqMan PCR, 13 virus isolation, 4 ELISA, 3 in situ hybridisation, and 3 histopathology, whereas clinical pathology was often the basis of diagnosis. Fifteen countries participated in the KHV PCR ring test of CEFAS (UK). Only 2 of 39 countries were not interested in future KHV ring tests. KHV was detected by PCR in goldfish in Italy (no clinics), and in many other fish species without clinics in Poland. Latency: Some experience with it in Germany (fish infected

with KHV >2 years), Japan (KHV in brains of surviving fish could be detected 1 year after experimental infection), Poland, and UK (at least KHV persistence, possibly latency. Measures varied: stamping out and disinfection, stop fish movements, and/or water temperature raised to 28-30°C, and/or vaccination (Poland tests vaccine with application of immunostimulants). Vaccination: Hong Kong uses a KHV vaccine, Poland and Israël the attenuated Israëlian vaccine, Germany reports no official use, Poland tests a new generation of vaccine. Many vaccinated koi are imported into the EU from Israël. Research on KHV: KHV for biological control of carp (Australia), epidemiology (China, Indonesia, Philippines, Poland a.o.), diagnostics (many countries, test antiviral products and test KHV resistant carp lines (Poland), assess impact on wild and fishery carp (UK), among many other subjects. KHV is notifiable at state or country level in 10 countries, but in most cases culling is voluntary. Training on KHV diagnostic techniques was needed.

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

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Comments:
S. Bergmann: *Nobody in Poland is using the vaccine.*
B. Hill: *There are two reference laboratories, one at Cefas and one in Japan.*

Koi Herpesvirus – sampling, diagnosis and results of the 2006 PCR ring trial

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Abstract: The past decade has seen the emergence of koi herpesvirus (KHV) as the cause of serious mass mortalities in koi and common carp around the world. Since first isolation in 1998 the virus has spread rapidly through global international trade in ornamental carp and has caused major economic losses in Israel, Europe, the USA, Japan, South-east Asia and South Africa. The virus is slow to grow and can be difficult to detect using cell culture hence a variety of PCR based detection methods have been developed and published. At the 2004 International Workshop on KHV the need for standardisation in KHV detection methods became apparent and a research study was undertaken at Cefas. The available published PCR protocols for detection of KHV were compared for sensitivity and specificity with PCR protocols based on protein coding regions of the KHV genome. Tissue sampling, extraction and amplification protocols were then optimised for the most effective assays. Adapted primer sets, targeting smaller sequences of KHV genome, were also tested and found to be more reliable in detecting the degraded DNA found in decomposed tissue samples. From these studies a standardised protocol was developed and the Bercovier-TK and modified Gray SpH primer sets were selected as the most robust for detection of KHV DNA in a range of tissue samples. This protocol was

adopted as the standard at the Cefas Weymouth laboratory. Other laboratories around the world were then contacted to request their participation in a ring-trial to assist in the validation of the protocol. Laboratories were asked to incorporate the selected primers in their current KHV PCR protocol using their usual assay parameters and compare them with the primers that they currently use. Alternatively, they could adopt the standardized protocol used at Cefas Weymouth. KHV-spiked tissue homogenates were sent to 21 laboratories from 19 countries around the world - a summary of the results will be presented.

Notes from the presentation:
The DNA-extraction methods tested were an in-house proteinase K method, AquaPure (BioRad), Easy-DNA (Invitrogen), DNAzol (Invitrogen) and DNEasy (Qiagen), which were all tested on infected gill tissue using Gilad and Gray SpH primers. Of these, DNAzol was chosen based on performance, cost and time.

Kidney, gill, spleen and gut and fresh, frozen and ethanol fixed tissues were evaluated with the following results:
Gill > kidney = spleen = gut.
Fresh = snap frozen > ethanol fixed.

Gilad, Gray *SpH*, Gray *Bam*H1, CNGV, Berkovier TK, Helicase, Tricapsid and Reductase primer sets were tested for comparative sensitivity. Berkovier TK and a modified *Sp*H (209 base pair product reduced to 151 bp) were chosen as the most sensitive.

Twenty-one laboratories participated in the KHV ringtest, using Berkovier TK, Modified-SPH, Gilad or Gilad-taqmann, with the following success rates: Berkovier-TK: 85%, Mod-SPH: 66.6% and Gilad: 50%.
Twelve labs used extraction kits, with 100% success for Bercovier-TK, 77.7% for Mod-SpH and 71.4% for Gilad. Nine labs used DNAzol, having 66.6% success with the Bercovier-TK, 55.5% with the Mod-SpH and 33.3% with Gilad.
Problems encountered by the labs included: Band in unspiked –ve control and smearing and extra bands with modified SpH-primers, KHV DNA was not amplified with one of the primer sets, and problems with the DNAzol extraction method.
Future work includes a 2007 ring trial and working towards being able to detect sub clinical disease.

Comments:
S. Madsen: *We participated in the ring trial and liked it because it validated our work, but we found a ghost band that came out as carp in a BLAST search. We used the TK-primers.*
R. Paley: *The TK was specifically designed not to react to carp, and we have never seen that.*

Confirmation of Spring Viraemia of Carp virus

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Abstract: Spring viraemia of carp (SVC) is an acute, haemorrhagic disease of several carp and other cyprinind species caused by the SVC virus (SVCV). SVCV is list III pathogen (Annex A, Council Directive 91/67/EEC) and is notifiable to the Office International des Epizooties (OIE). Pike fry rhabdovirus (PFRV) is the causative agent of red disease, an acute condition characterised by haemorrhagic lesions on the trunk, ascites and high rates of mortality, primarily in the fry of northern pike (*Esox lucius* L.1766) (de Kinkelin, 1973; Fijan 1999). Rhabdoviral isolates, initially identified as PFRV by neutralization testing, have also been made from other fish species including grass carp (*Ctenopharyngodon idella Val.*) tench (*Tinca tinca* L.), white

bream (*Blicca bjoerkna* L.), top-mouth gudgeon (*Pseudorasbora parva*) brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*); roach (*Rutilis rutilis*), golden ide (*Leuciscus idus* var. *auratus*); sheatfish (*Siluris glanis*) and common bream (*Abramis brama*).

Phylogenetic analysis based on a 550 nucleotide partial glycoprotein gene sequence of putative SVCV and PFRV isolates identified two further potential species of cyprinid vesiculotype virus. These viruses were described as Grass carp rhabdovirus (GrcRV) that is represented by isolate V76, and tench rhabdovirus (TenRV), which is represented by isolate S64 (Stone *et al.,* 2003)

It has proved very difficult to distinguish SVCV from the other vesiculotype viruses using serological tests based polyclonal antibody because the antibodies directed against SVCV will cross-react to various degrees with PFRV, TenRV and GrcRV. Serological tests based monoclonal antibody are generally more specific for SVCV but they fail to detect many of the SVCV isolates, in particular, those of Asian origin.

The OIE recommended test for confirmation of SVCV is based on degenerative primers that will amplify partial G-gene sequences from the full spectrum of SVCV isolates. Confirmation of SVCV is then achieved using BLAST to compare the amplicon sequence with the nucleotide sequences deposited with GenBank and EMBL databases. The OIE recommended primers will also amplify the equivalent region from TenRV, but this virus can be considered as non-SVCV based on the results of previous phylogenetic studies and it is not notifiable to the OIE.

Notes from the presentation:
SVC is found in European countries with low water temperatures during winter, and in east European countries, the United States, Canada and the Peoples Republic of China.
The primers for the RT-PCR were designed by first identifying annealing sites by alignment of published amino acid sequences for the SVC glycoprotein and vesicular stomatitis virus, and then designing primers to anneal to the regions encoding the conserved amino acids, using the published sequence for SVCV. These primers are able to detect all tested SVCV isolates so far, but they also replicate tench rhabdovirus. In semi-nested PCR using 30 cycles it is possible to detect <10 virus particles per mg of fish tissue.
Cell culture is still the OIE standard. If CPE occurs, identification procedures outlined in the OIE manual should be undertaken immediately. Use RT-PCR and post-amplification hybridisation as an alternative to sequencing, but when in doubt: treat suspect cases as SVC and send them to Cefas for confirmation.

Comments:
P. Stylianou: *Have you seen SVC in higher temperatures?*
K. Denham: *No it is unusual to see it at temperatures higher than 15 degrees.*
H. F. Skall: *Why do you grow it at 20 degrees?*
D. Stone: *Details can be obtained from Keith Way from Cefas, but my understanding is that the virus culture is more reproducible at 20 degrees.*
S. Bergmann: *Normally SVC grows better at 20 degrees.*
N. J. Olesen: *Now that SVC is a listed disease we would like to have uniform identification procedures. Could we make that for all the NRLs within the next 6 months?*
D. Stone: *There is a standard identification protocol described in the OIE diagnostic manual. A reverse hybridisation assay is due to be published in Diseases of Aquatic Organisms later this year and could be released earlier. But for now you need to perform sequence analysis to discriminate between SVCV and TenRV.*
N. J. Olesen: *That is probably not possible for all the 30 laboratories.*
V. Jurgelevicius: *We need exact protocols.*
D Stone: *Fully detailed protocols for SVCV identification are given in the OIE manual.*

Detection of *Campylobacter* in poultry- aspects of diagnostic PCR

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Abstract: The PCR technique is now widely used for detection of food borne pathogens to overcome the limitations of conventional microbiology procedures. PCR has successfully been applied to detect *Campylobacter* spp. in poultry, food and environmental samples.
The production of certified, fresh *Campylobacter* free poultry product has been taking place since 2002 and is the world's first example of fresh chickens labelled for sale as *Campylobacter* free.
This is done by the use of separate slaughtering of *Campylobacter* positive and negative flocks made possible by surveillance of the *Campylobacter* status in poultry flocks before slaughter; and by the use of a 5 hours PCR testing scheme on faecal swabs taken at the slaughterhouse.
The short analysing time is obtained by the use of a semi automatic DNA purification method, a pre-made PCR mix and handling of samples with multi channel pipettes during most of the steps in the procedure.
To make a robust PCR method several aspects should be considered. This includes primer specificity, sensitivity of assay and also the reliability of the method. The latter involves validation of the repeatability as well as assessment of detection limit in association with the detection probability.
One potential problem of PCR is failure of DNA amplification due to the presence of inhibitory substances that partially or completely prevents the PCR reaction resulting in false negative results. To exclude false negative PCR reactions an internal control of the PCR reaction should be included
either by adding primers specific for a gene present in all specimens or by adding synthetic DNA with primer regions identical to target DNA to the master mix. Alternatively, a control organism may be added to the sample before DNA extraction. For all methods it is crucial to ensure that the internal control has a comparable sensitivity towards inhibition as has the target DNA.
Finally, to obtain a DANAK (The Danish Accreditation and Metrology Fund) accreditation, documents for tracking of materials, instructions for methods, maintenance of controls and for calibration should be available.

Notes from the presentation:
The presentation describes the use of swabs for use in PCR diagnostics. Non-lethal testing is an important topic for future fish health surveillance. The experiences from the development and validation of a swap technique for detection of Campylobacter might be very useful in this aspect.
Samples can be taken either by washing the chicken, cloacal swabs or from faecal matter.
The swab is an easy and fast method and there are many types of swabs.
When the samples are received, *Y. ruckeri* is added for internal control of PCR. Magnetic beads are used for DNA-extraction before the PCR which is performed using frozen prepared mastermix.
Aspects of the PCR assay:
It has turned out that transport media is not as good as transporting in water, and the swabs can also be transported in empty tubes.
Isolation of DNA can be done using automated methods like magnetic beads or by hand.
The mastermix needs to be selected individually for each specific PCR-test, and often needs optimization. The primers also have to be specific, and this should be tested in practice.

An internal control such as bacteria/virus, bacterial/viral DNA, plasmid, PCR product or a synthetic DNA product should be used, and tested so that it works as it should, and does not disappear with inhibition or is to strong. Both internal control and primer should work in the presence of inhibitor.
Confirmation of bands can be done using sequencing, hybridisation on membrane, PCR-ELISA or Nested PCR.

For validation, the detection limit of the PCR assay compared to culture was determined, and the diagnostic sensitivity and specificity was determined comparing the PCR with cell culture.
The test was also run by two different persons, in order to test the repeatability and the kappa value was satisfactory.
Finally, the test was accredited by DANAK, which ensures repeatability and quality of the test.

SESSION V: SCIENTIFIC RESEARCH UPDATE

Chair: Guiseppe Bovo

Vertical transmission of infectious salmon anaemia (ISA)

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Abstract: Infectious salmon anaemia (ISA) is a viral disease that was first recorded in 1984 in fish from an Atlantic salmon hatchery in Norway. The disease is characterised by a circulatory collapse including severe anaemia, hence the name of the disease. The properties of ISA virus are consistent with those of the *Orthomyxoviridae*, and ISA virus is now classified as the type species of the genus *Isavirus* within this virus family. ISA disease outbreaks have only been found in Atlantic salmon, though the virus has been found in both farmed rainbow trout and coho salmon, and in wild brown trout.

From 1984, the ISA epidemic increased steadily in Norwegian salmon farming and peaked in 1990 when ISA was detected in 80 fish farms. At present, approximately 440 ISA outbreaks have been registered in Norway and of these, only three (0.7%) have occurred in the fresh water phase. During 1988-1991, a number of regulatory actions were implemented to control the disease. The aim was to interrupt the transmission of infection and to reduce infection pressure. The results of these actions, together with significant improvements in husbandry practice, were a remarkable and rapid reduction of ISA outbreaks. During the last 10 years, the annual number of ISA outbreaks have varied from 3-20. However, the Atlantic salmon industry develops and changes continuously, and both structural and management changes may have impact on disease control issues.

To ensure that the Norwegian Food Safety Authority bases its management on internationally accepted knowledge, the Norwegian Scientific Committee for Food Safety was in 2006 asked to consider several questions related to ISA disease management. These questions also included questions related to vertical transmission, i.e. a) Can ISA virus transmit vertically? b) How high is the probability of spread of the agent and/or development of disease, as a result of vertical transmission? To elucidate these questions, the Scientific Committee established an *ad hoc group* consisting of national and international experts representing Scotland, Canada and the USA. Both published experimental data, unpublished data and data based on field experience were considered.

The conclusions drawn were as follows: "A) The ad hoc group is of the opinion that vertical transmission of ISA virus cannot be excluded. However, available data are inconsistent, and there was disagreement within the ad hoc group on the interpretation of the available data. B) The probability of spread of the ISA virus as a result of vertical transmission may depend on individual characteristics, such as clinical status and virus titre in the parent fish, intracellular or extra cellular transmission, which is regarded as the dominant route; and/or strain characteristics of the virus. The probability of further spread via eggs, fry or smolt as a result of vertical transmission will depend on the efficacy of intervening management procedures, such as disinfection and prophylactic treatment post-stripping.
Present relevant knowledge is scarce on these variables. It is not possible from the available information to estimate the probability of spreading of the agent through vertical transmission.
Spread of the disease as a result of vertical transmission may be regarded as a consequence of ISA virus being vertically transmitted. The low number of outbreaks in the fresh water stage (0.7 %) and lack of ISA disease in some countries that over the years have imported substantial numbers of eggs from Norway, suggest that the probability of disease emergence following vertical transmission of virus is low."

The complete report from the *ad-hoc group* can be downloaded from the following WEB-site: http://www.vkm.no/eway/default.aspx?pid=266

Notes from the presentation:
For characterisation of ISA, phylogenetic studies has been performed based on sequencing the HE-gene by Nylund et al. in 2007, showing that there are very small differences within Europe-G2
The definition of vertical transmission by the OIE means the transmission of a pathogen from a parent aquatic animal to its progeny via its sexual products.
Vertical transmission has been described in the literature by: Thorud and Djupvip et al. 1988, Melville and Griffiths et al. 1999 and in a report by Søfteland et al. 2005 but none of the authors have been able to detect vertical transmission.
It has been described by Nylund et al. 2007 that the major transmission route of ISA virus in Norwegian aquaculture is vertical transmission. The conclusions were based on phylogenetic studies sequencing the HE-gene from many outbreaks in Norway and from sub clinical infections. The samples investigated originated from fish at sea sites, broodfish and smolt.
A report from EU concluded that there is no any hard evidence for vertical transmission of ISA virus and vertical transmission was regarded to insignificant in the epidemiology of the infection (Bovo et al. 2005).

In summary vertical transmission of ISA virus cannot be excluded but the probability for spread of ISAV through vertical transmission has been considered low. Finally the probability of development as result of vertical transmission has been considered low.

Comments:
N. J. Olesen: *Would it be safe to import eggs from an infected area?*
K. Falk: *Yes, I think so when you have good surveillance of broodstock. By screening you will have positives – but are they real positives?*

PANDA: Diagnostic methods of disease hazards to European aquaculture

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Abstract: Within the EU PANDA project (www.europanda.net), based on a list of most important exotic, emerging and re-emerging (non-exotic) disease hazards to aquatic animals in the EU identified, current diagnostic methods for the identified diseases of fish, molluscs, crustaceans and amphibians were investigated. The most important diagnostic methods for screening and confirmation are presented per hazard, and the set up of the final report. The official report of this PANDA work package is due September 2007, and will be distributed to all NRL's of Fish Diseases, a.o.

Table: Rough PANDA WP4 results:
Fish disease/pathogen list, and appropriate diagnostic method(s) for screening and confirmation respectively (for references, please see the panda-website): ab = antibody; ag = antigen; E.M. = electron microscopy; ex = exotic to the EU; histo = histopathology; ICC = immunocytochemistry; IFAT = immunofluorescence; IHC = immunohistochemistry; IPMA = immuno peroxidase monolayer assay; ISH = in situ hybridization; LAMP = loop-mediated isothermal amplification; n-ex = non-exotic to EU; RFLP = restricted fragment length polymorphism; SN-test = serumneutralisation test; VI = virus isolation.

Fish disease/pathogen	Screening method	Confirmation methods
EHN (ex)	Clin.pathol., VI, IFAT, IPMA, ELISA (ag & ab); some are ISO9001	IFAT, IPMA, ELISA (virus & serol.), SDS-page; PCR, IHC some are ISO9001
RSIV (ex)	Clin.pathol., VI,	IFAT (ISO); IPMA; sequencing; PCR; LAMP; histo; IHC; E.M.
ISA (ex)	Clin.pathol., VI, RT-PCR, haematology; *(not definite: comparison of tests to be included)*	RT-PCR; IFAT; IPMA; histo; ISH; ELISA (ab); haemabsorption; SN-test; E.M.
KHV (n-ex)	Clin.pathol., VI (low sensitive); ELISA (ab); PCR & RT-PCR; LAMP	IFAT (after cpe and with kidney imprints); ELISA (ag & ab); PCR & RT-PCR; sequencing; histo; ISH; LAMP; E.M.;
Strept.agalactiae (ex)	Clin.pathol.; isolation;	Clin.pathol.; isolation; biochemical typing; serology; PCR; DNA sequencing; DNA-DNA hybridization; Sherman criteria (some are validated)
Strept.iniae (n-ex)	Clin.pathol.; isolation;	Clin.pathol.; isolation; biochemical typing; serology; PCR; DNA sequencing; DNA-DNA hybridization; Sherman criteria (some are validated)
Lactoc.garvieae (n-ex)	Clin.pathol.; isolation;	Clin.pathol.; isolation; biochemical typing; serology; PCR; DNA sequencing; DNA-DNA hybridization; Sherman criteria (some are validated)
Trypanopl.salmositica (ex)	Clin.pathol. (suspicion); Antigen-capture ELISA; Antibody capture ELISA	Fresh preparate and fixed smear of mucus/fluid (standardized); Haematocrit centrifuge technique (standardized, highly sensitive); IFAT (ab); MISET (ab); Antigen-capture ELISA (standardized, highly sensitive); Antibody capture ELISA (standardized)
Ceratomyxa shasta (ex)	Clin.pathol. (suspicion); isolation; PCR; quantit.PCR;	fresh preparate (standardized); fixed smear; isolation; IFAT (ag); IPMA; PCR (standardized); quantitat.PCR

	non-lethal PCR	(standardized, very sensitive); histo (standardized); IHC; ISH (standardized); non-lethal PCR
Parvicapsula pseudobranchicola (ex)	Clin.pathol.; parasite isolation	parasite isolation; sequencing; PCR (highly sensitive); histo; ISH; ….
Neoparamoeba pemaquidensis (ex)	Clin.pathol.(standardized at farm level); gill histo (standardized & validated)	gill histo (standardized & validated); fres preparate; fixed smear (standardized and validated); parasite isolation and identification (stand.&valid.); IFAT (ab) (stand.&valid.); immuno dot blot of mucus (standard.); sequencing; PCR on clonal cultures; ICC (stand.&valid.)
Gyrodactylus salaris (n-ex)	Clin.pathol.; isolation; morphometry; RFLP	morphometry; sequencing; RFLP
Aphanomyces invadans (EUS) (ex)	Clin.pathol.; isolation; PCR	Fresh preparate; fixed smear; ELISA (ab); Western blot; haemagglutination; sequencing; PCR; histo; IHC; ISH; E.M.; pyrolysis mass spectrometry

Acknowledgements
Many international experts are thanked for their assistance in providing information to obtain the table above. They are mentioned at the PANDA website, and will be fully acknowledged in the final report, due Sept 2007.

Notes from the presentation:
The WP4 of the PANDA project consists of the following members; Olga Haenen (WP4-leader, fish pathology, parasitology, virology and QA), Inger Dalsgård (fish bacteriology), Niels Jørgen Olesen, Britt Bang Jensen, Ellen Ariel (fish virology and amphibian diseases), Jean-Robert Bonami (crustacean diseases) and Jean-Pierre Joly, Isabelle Arzul (shellfish diseases).

The deliverables for WP 4 included reporting on the current best methods for rapid and accurate detection on main diseases and requirements for improvements and for eventual standardization, validation and harmonization throughout Europe.
WP 2 defined which exotic fish diseases including viruses, bacteria's, parasites and other diseases as mollusc diseases, crustacean diseases and amphibian diseases should be looked into.
Tables describing the current methods in literature can be found at PANDA's web page. In the final report (in prep.) a chapter for each pathogen has been made including a short description of the pathogen/disease, screening techniques, confirmatory techniques, what to do if disease is suspected, the EU legislation and OIE recommendations.
For the crustacean diseases there is only one laboratory in Europe (Montpellier) able to make diagnosis of diseases and there are no NRL's or CRL's. The working group has recommended appointing the viruses on the EU list.
In general training was needed on points such as risk analysis, sampling strategies, screening analysis and developing Standard Operating Procedures. Furthermore training was still needed for many of the present diseases.

Comments:
V. Jencic: *What happens with the project when funding ends?*
B. Hill: *We will look at some funding to continue the project, since we need to come together for meetings but we don't know anything yet.*
S. Cabot: *If there are no NRL's or CRL's yet the laboratories must apply for it before May 2008.*

B. Hill: *There are more than 1 lab able to diagnose crustacean diseases, for instance CEFAS is able to diagnose them. The problem of no CRL yet, will be solved by the new Directive 2006/88/EC, in which the EC must appoint a CRL on crustacean diseases by May 2008.*

Highlights from the DIPNET Project - Disease interactions and pathogen exchange between farmed and wild aquatic animal populations – A European Network

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Abstract: The presentation provides an overview of the DIPNET project which was carried out with support under FP6 of the European Commission. DIPNET provided scientific reviews of current knowledge, stimulated the exchange of information, identified needs for future research and identified scientific approaches to investigate diseases in aquatic environments.

Work Package 1: Review of disease interaction and pathogen exchange

WP1 produced a literature review of disease interactions and pathogen exchange between farmed and wild aquatic animals. Two workshops involving over 100 participants were held to facilitate information exchange, discussion and the production of a scientific report with bibliography.

Work package 2: Risk assessment and modelling of pathogen exchange

WP2 produced a scientific review on risk assessments and modelling in aquatic animal health. An introductory risk assessment seminar, specifically targeting potential users was held.

Work package 3: Infectious disease epidemiology in wild populations

WP3 made a review of current activities and methods for fish disease epidemiology, and a corresponding seminar was organised.

Work package 4: Network building and knowledge dissemination

WP 4, focussed on network building and knowledge dissemination, the production of a project web page with scientific bibliography, as well as approximately 100 project leaflets, posters or presentations.

Work package 5: Project management

WP5 provided scientific project co-ordination and liaison with the policy-makers of the European Union. Besides administrative deliverables, a document containing a proposed policy implementation plan (PIP) was produced.

Further information and the outputs from DIPNET can be found at http://www.dipnet.info/

Notes from the presentation:

DIPNET was a 2-year project (2004-2006), the purpose of which was to strengthen current knowledge about disease interactions and pathogen exchange and disseminate this to scientists.

A report of the review of disease interactions and pathogen exchange between farm and wild finfish and shellfish in Europe is available on the webpage and includes chapters on disease scenarios in the North Atlantic, continental Europe, and Mediterranean. For the North Atlantic there is evidence that transmission from wild fish can be important in disease emergence in aquaculture, but limited data exists implicating disease transmission from aquaculture to wild fish. In continental Europe it is thought that transfer from wild fish plays a larger role where

control programmes are in place. For the Mediterranean region, there were less information available, but nodaviruses are considered important, and there are special considerations since wild fish are caught for ongrowing and unprocessed wild caught fish are used as feed for tuna.
For shellfish and crustacean diseases there is circumstantial evidence of pathogen exchange both from wild to farmed populations and vice versa, but no scientific evidence demonstrating the underlying mechanisms is available.
It is difficult to investigate pathogen exchange between farmed and wild fish because there are very few barriers between the two populations. Furthermore, there is limited information on the health status of wild fish, and even though farmed fish are surveyed, there is a lack in validation of the diagnostic assays.
DIPNET has made a review of disease monitoring in Europe, of epidemiological surveillance in wild aquatic populations and of causality of transfer of pathogens between wild and farmed aquatic animals.
Information is available until November 2008 on www.dipnet.info. The website has 530 subscribers, and 46 newsletters have been published

There are still many gaps in knowledge of the health status of aquatic animals, so development and evaluation of diagnostic methods, development of epidemiological and risk assessment methodology and demonstration of causality and impacts are recommended.

Vaccination against VHS in rainbow trout: Experimental testing and perspectives related to practical fish farming.

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Abstract: European production of farmed rainbow trout frequently encounters considerable problems with diseases caused by viruses. No treatment is available, and with few exceptions, this also counts for vaccines. One of the most important diseases is caused by the rhabdovirus viral haemorrhagic septicaemia virus (VHSV). Outbreaks of VHS can result in very high mortality among rainbow trout of all sizes, and the only available control measure is presently stamping out of infected farms in combination with intensive surveillance and control programmes.

Killed and attenuated virus can be used for vaccination, but is expensive to produce or not suitable for use under field conditions respectively. A new and promising prototype DNA vaccine against VHS in rainbow trout has recently been developed. The purified vaccine is non-infectious and cannot replicate in the fish. Upon intramuscular injection, a single vaccine dose can provide high, rapid and long lasting protection of rainbow trout fingerlings against VHS under experimental conditions (Fig. 1). The vaccine could be a valuable tool for reduction of losses caused by the virus under farming conditions. This includes not only regular use as prophylaxis against mortality in endemic zones but also transient use in connection with eradication programmes where stocking with vaccinated fish in exposed areas one or two seasons prior to disinfection of the farms could help to reduce circulating amounts of virus. Although development of DNA vaccines have been attempted for a number of pathogens in a number of different fish species, the DNA vaccines against salmonid rhabdoviruses such as VHSV remain the most efficient and also the most extensively analyzed to date. The vaccines are highly efficacious under a variety of experimental conditions including different fish life stages, different salmonid host species, and against challenge with different virus strains

A preliminary testing of the VHS DNA vaccine under field conditions has recently been conducted in Denmark. The fish were vaccinated during the winter at farms without VHS and

were transferred to net-pens on farms with ongoing VHS outbreaks. The results of the experiments will be summarized.

As with other types of vaccines, a number of safety aspects must be considered in relation to DNA vaccines. Since DNA vaccines are produced by molecular biotechnology, their use in fish and other husbandry animals require acceptance not only by the authorities but also by the consumers in general. These aspects will be shortly discussed.

Fig. 1

The diagram below illustrates how the protection induced by DNA vaccination against VHS and IHN in rainbow trout is a combination of rapidly activated general anti-viral mechanisms followed by more slowly induced specific and long-lasting immunity.

Notes from the presentation:

For creating a DNA vaccine against VHS, the gene encoding the surface glycoprotein (G) of the VHS virus has been cloned into a bacterial plasmid which can be produced easily in E-coli. The live bacteria harbouring the plasmid are considered as genetically modified organisms (GMOs) but after purification the plasmid is not alive and therefore not classified as a GMO.

The vaccine is injected directly into the fish by intra-muscular injection. The plasmid is taken up by a small number of cells and expression of the viral G protein by these cells induce a protective immune response in the fish.

The advantages of the DNA vaccine are that there is a high safety and no risk of disease. Furthermore the protective effect is rapidly established and long lasting.

The disadvantages are that injecting is not practical for commercial use in small fish and the licensing is expensive. Also, the consumer's acceptance of use of this type of gene technology in production of healthy animals is important in some countries.

Under experimental conditions, it has been demonstrated that the vaccine protects against challenge with VHS virus already 4 days post vaccination and that this effect is due to activation of non-specific antiviral interferon-related mechanisms. After 3-4 weeks a specific and long-lasting immunity to VHS is established, correlating with occurrence of virus-neutralizing antibodies. The vaccine has been tested under field conditions in a small-scale pilot experiment, in which the fish were not allowed to go into the food chain. Vaccinated and control fish were kept in cages, which were put into ponds at farms with VHS outbreaks. In some cages a highly significant protection was observed, whereas others showed no difference between vaccinated and control fish. Concerning the latter, it appeared that the cage-setup occasionally gave favourable conditions for outbreaks of other diseases. Although compromised by occurrence of other diseases, the results suggested that the vaccine can protect against VHS also under field conditions. Further testing in up scaled experiments including vaccination of all fish in whole ponds is needed to fully evaluate the potential of use of the DNA vaccine for elimination of VHS outbreaks.

Comments:

A. Prapas: *Have you tried to include adjuvant?*

N. Lorenzen: *Yes, but the DNA vaccine seems to have a built inn adjuvant effect.*

R. Raynard: *Is it possible to change the opinion among the costumers so that the fish can be sold?*

N. Lorenzen: *An important point is that the authorities do not require labelling of vaccinated fish since this will make consumers avoid buying the product.*

R. Raynard: *If the supermarkets will buy the fish the consumers will maybe buy the fish.*

A. Kvellestad: *What is the risk in terms of food safety?*

N. Lorenzen: *There is a chance that residual vaccine can be taken up by a few cells in the consumer, but the risk of negative side effects is very little. Several clinical trials have been*

conducted with DNA vaccines in humans during the last 10 years, and no negative side effects have been reported so far.
T. Wahli: *Will vaccinated fish become carriers following exposure to VHS virus?*
N. Lorenzen: *Yes, some of the vaccinated fish will get infected, but to a lower level compared with non-vaccinated survivors of VHS.*

SESSION VI: Update from the CRL
Chair: N.J. Olesen

Protocol for management of underperformance/lack of collaboration of National Reference Laboratories (NRLs) in comparative testing and lack of collaboration with CRL activities

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Draft: According to article 32 of Regulation (EC) 882/2004, Community reference laboratories (CRLs) shall be responsible for coordinating application by the NRLs of analytical methods, in particular by organising comparative testing and by ensuring an appropriate follow-up of such comparative testing.
Article 33 of the Regulation establishes that NRLs shall collaborate with the CRLs in their area of competence.
The NRLs are a key tool for the proper implementation of official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules, therefore their performance is of outmost importance.
Appropriate actions must be taken if the results of comparative tests reveal underperformance or if NRLs fail to collaborate properly with the corresponding designated CRL.
The following two-step protocol is suggested in case of
a. underperformance (i.e. failure in proficiency test)
b. lack of collaboration by the NRLs with the CRL:

Phase 1

a. underperformance (i.e. failure in proficiency test)
- CRL should contact the NRL and provide assistance trying to identify the origin of the bad result. On the spot visits and training could be foreseen if necessary.
- Repetition of the comparative test if feasible (e.g. within 3 months) and close assessment of the results by the CRL

Confidentiality should be kept during this phase in order to ensure good co-operation from the NRL. The results of the PT and the codes of the laboratories are included in the report transmitted to the Commission. Apart from that there is no need to further involvement of the Commission until the results of the following comparative test are available and re-assessed.
b. lack of collaboration by the NRLs with the CRL:
- CRL should contact the NRL if lack of collaboration with CRLs activities. CRL should ask the NRL for the reasons of no participation to a proficiency test or a workshop. the justification provided by the NRL should be included in the report submitted to the Commission

Phase 2

a. underperformance (i.e. fail in proficiency test)
- If the results of the following comparative test still reveal underperformance of the NRL or the collaboration of the NRL is not adequate, the Commission shall be informed officially by the CRL including a report of the main findings and corrective actions to improve the situation.
- The Commission shall inform the competent authority and require that appropriate actions are taken.

b. Continuous lack of collaboration by the NRLs with the CRL:
- In case of repetitiveness of the lack of response of the NRL, the Commission shall be informed officially by the CRL and the Commission shall inform the competent authority and require that appropriate actions are taken.

Notes from the presentation:
The proficiency test supplied by the CRL has consistently shown that the same laboratories have been unable to correctly identify the notifiable diseases. Therefore training of laboratories was included in the work programme for 2006. In order to make the best training programme for each laboratory, Nicole Nicolajsen and Ellen Ariel went to 5 laboratories, and went through a fact-finding mission ending up with a proposal of a training schedule for each.
As part of the training programme, a workshop was held for two persons from each of the five labs. There were two parallel programs; one in cell culture and one in identification, and there were also lectures on how to solve the proficiency test.
Afterwards, a follow-up mission was performed for each of the laboratories, where Nicole visited each lab, going through how the new knowledge had been implemented, and helping them receive and initiate a comparative test that was received by the lab while Nicole was there. This time, everybody solved the ringtest correctly. Later, a second ringtest was provided which was also solved correctly by all the five laboratories. So the conclusion is that this is a very good method to help the laboratories and make sure they can perform the tasks assigned to them.

The commission has made a draft on a protocol for management of collaboration/lack of collaboration of the NRLs in comparative testing and collaboration with the CRL, which can be found in the booklets. We do not know when this protocol will be implemented.

Comments:
S. Cabot: *The draft has not been adopted yet, but some of the duties are already described in the 2006/88.*
N. J. Olesen: *We used the money that normally is spent on the ringtest on this training programme for these five laboratories. It has been a huge task, and the amount of time needed was a surprise to us. So we cannot offer both this amount of traning and a ringtest.*

Report from year 2006

The reports and work programmes for the CRL was presented by Niels Jørgen Olesen.
The Technical Report 2006 is included in the folder.
One of the big tasks in 2006 was the training of five laboratories in diagnosis of viral fish diseases, and the validation done by Sanne Madsen (presented at this meeting). The characterization of virus isolates is also a large task, and we thank the NRLs for providing the isolates.
A big recurring task is the organization of the Annual meeting, where the focus in 2006 was on characterisation and definition of pathogens. The Survey & Diagnosis 2005 was sent out, after modifications agreed upon at the previous AM.
This year, the focus has been on the new Council Directive 2006/88. In annex VI of the Directive you will find the functions and duties for the CRLs and the NRLs.
One of the serious changes is that the NRLs have to have a QA-system in place, which might be a big task for some of the laboratories, but it is required by the EU in order to make sure that they can trust the results from the laboratories.

Work programme for 2007

This can be found in the booklet. The tasks include producing a report from this AM, which hopefully will be finished by august 1st.
Questions on the occurrence of the new listed diseases were included in the S&D.
In order to update and maintain the library of virus isolates, we are working on implementing a database with the help of the EPIZONE-project.
Another task is the supply of antisera and standard reagents for the NRLs.
This year we are providing an inter-laboratory proficiency test and will collate and analyse the data obtained. We need input on what should be included in this proficiency test. The proficiency test will be provided in the fall. The test will be on the ability of the laboratories to detect VHSV, IHNV, and SVCV. The difficulty is how to include KHV and ISAV. One option could be to ask Cefas, Weymouth, to provide the ringtest for KHV, while the OIE reference laboratory for ISA in Oslo might help providing a test on ISA. It is very important for the CRL to collaborate with other laboratories in order to supply these ringtests.

Comments:

B. Hill: *The new directive does not state which diseases it is that the CRL should consider, is it just assumed that it is the five listed diseases? Also, there is an obligation to beware of the exotic diseases.*

S. Cabot: *Article 55 states that the CRL should work with the diseases connected with this directive. For exotic diseases it does not say much.*

N. J. Olesen: *You are all very welcome to contact the CRL if you are interested in training or other support.*
We have not done much work on the webpage, but this will be one of the main focus areas of 2008. We would like to be able to link to other projects. There is a confidentiality issue, where we need to make sure that no confident material is made public.
How do you feel about the report from the AM being made public?

B. Hill: *There could be a section that is open to all NRLs, where there could even be a forum.*

C. Crane: *for the mollusc CRL, there is a public section and a section for NRLs, where you can find SOPs.*

O. Haenen: *There could also be leaflets on diagnosis and presentations from this meeting. But the website needs to be updated, or nobody will look at it. E-mail alerts are imperative.*

B. Hjeltnes: *We should give it serous thought. Who is the information for? Universities, farmers..?*
We should be careful with the report from this meeting being published all over, because it might be misused or misunderstood.

N. J. Olesen: *Is this webpage a high priority? In many projects, webpages are created for a short span of years, but it would be good to have this, because the CRL is continuous.*

B. Hill: *Perhaps just the cosmetic improvements should be made for now, and then ideas for the design and function should be presented at the next meeting.*

R. Raynard: *Would it be possible to formulate what is the cost of running the CRL and make a business plan, where it is possible to see the costs of each task, perhaps making a draft before the next meeting, so people can have a say.*

N. J. Olesen: *We receive 150.000 euros pr. year, which makes us one of the cheapest CRLs in the Community.*

R. Raynard: *The CRL does a good job, and we get good value for the money!*

S. Cabot: *Would it be possible to merge the PANDA website and the CRL webpage? And could the objectives of the PANDA and the CRL merge?*

C. Crane: *This has been done by the mollusc CRL.*

N. J. Olesen: *The purpose of PANDA was to enlarge the NRL network. In the beginning of the PANDA project it was suggested that the CRL could take over the PANDA.*

Work programme 2008

A first draft can be found in the booklet.
One objective for 2007/8 is to create a tool for molecular epidemiological tracing with help from the FP6 project EPIZONE. Another is to assess if all 5 listed non-exotic diseases can be surveyed by the same sampling plans and diagnostic methods as described i.e. in Commission Decision 2001/183.
A survey for other diseases such as PD, SD or VNN using the same procedures could be performed, and we suggest for the next annual meeting to have a workshop for diagnosing of EHN and EUS, hopefully with the help of the RANA-project, and people working with EUS.
Do you have any other ideas or suggestions on objectives?

Comments:
H. Hellberg: *We have shown that it is possible to use tissue samples from the VHS and IHN surveillance to make RT-PCR for ISA.*
K. Denham: *But the physical requirements for sampling are different, i.e. water temperature etc.*
B. Hill: *The sample size might also be very different. Perhaps you could produce a draft that could be circulated for the NRLS for comments.*
S. Cabot: *It is a good idea to look at cost-effectiveness; we need to find the most effective way of achieving the knowledge.*
O. Haenen: *It would be good to have information on all the new diseases on the website.*
N. J. Olesen: *There are already tutorials on the webpage, but we are unsure if they are used.*
H. Hellberg: *What about preparedness for emerging diseases? Including how to identify these, like a harmonisation.*
N. J. Olesen: *It is a whole research area. Both EPIZONE and RANA deal with emerging diseases.*
B. B. Jensen: *Should we have a follow-up on the risk-based surveillance at the next meeting?*
B. Hjeltnes: *We do not speed up the process by discussing it on the annual meeting.*
N. J. Olesen: *Since we are lab-people, we are not the ones doing it. But make sure that your own CA starts working.*
S. Cabot: *Actually the deadline for telling the EC how it will be taken care of is May 1st.*

Finally, it was suggested that the annual meeting next year should be held on **16-18th of June 2008.**
Where will be decided later, but it is likely to be held once again at VET-DTU in Copenhagen using workshop facilities at the lab or at the University near by. An alternative option is in Århus in a site close to our fish diseases laboratory where a practical workshop could be organised.