KOI HERPESVIRUS DISEASE (KHVD) SURVEILLANCE AND DIAGNOSIS

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Meeting on new recommendations for sampling, diagnosis and surveillance of KHV

- The European Commission gave permission for the EURL to organize an expert meeting in order to discuss (and agree !) common new recommendations for implementation in a new Commission Decision
- A 2 day meeting was held at the premises of the EURL, here in Frederiksberg, and three experts in the field of KHV from Germany, Netherlands and UK, were invited to participate
- The meeting was very successful and produced final drafts of two documents:
  - **The Commission decision Part 2 on surveillance and diagnostic methods for KHV**
  - **Diagnostic procedures for the surveillance and confirmation of KHV disease.**

Summary of recommended changes

- Significant changes from the former versions were accepted and recommended for inclusion in the commission decision. Among the changes are:
  - **The splitting of sampling and diagnostic tests for disease diagnosis and for surveillance purposes**
  - **Inclusion of real-time PCR as the method of choice for surveillance**
  - **Specification on how to define a CyHV-3 strain**.

Meeting agenda: Items for discussion

- Sampling procedures
- Susceptible species
- Molecular techniques for disease diagnosis
- Molecular techniques for surveillance
- Cultivation (cell culture isolation)
- Serology
- Other issues (e.g. CyHV variants)

It was agreed that there was need for establishing two separate chapters:

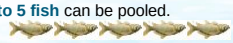
1) addressing surveillance and sampling & 2) diagnostic tests

Sampling procedures: General provisions

- Water temperature = >17°C - and sample no sooner than 2 weeks after these temperatures are reached.
- Optimal to sample fish that have been kept for a prolonged time period at the virus permissive temperature range (2-3 weeks at 20°C to 26°C).
- Certain management practises (e.g. netting and/or transport of the fish) can reactivate the virus in fish with a carrier status, thus increasing the chance of KHV detection.
- If possible, collect samples 24 hrs after such management practices to enhance the chance of KHV detection
  (Bergmann and Kempter 2011)

Sampling procedures: Tissue samples

- Gill and kidney tissue shall be sampled [in addition spleen, encephalon and intestine can be included]
- In **acute cases** tissue material of **up to 5 fish** can be pooled.



- Non-lethal samples – e.g. blood, gill swabs, gill biopsy, mucus scrape can be used in certain cases (e.g. very valuable fish, suspect case)
- **For surveillance**, pieces of gill and kidney tissue from a **maximum of 2 fish** may be pooled

Sampling procedures: sample storage, transport & processing

- For disease diagnosis and surveillance: fish can be sent alive or killed and packed separately in sealed aseptic containers
- Alternatively frozen organs (gill and kidney) or organ pieces preserved in 80 – 100 % alcohol (e.g. ethanol) or viral transport medium (to be processed within 48 hrs after collection) can be used for testing by PCR based methods
- Large tissue samples must be homogenized (e.g. mortar and pestle, stomacher) and subsamples retrieved for DNA extraction before clarification, alternatively subsamples can be collected from each tissue included in the sample and placed in “lysis-tubes”
- Acceptable tissue to medium ratio is 1 : 9 w/v. Minimum 25 mg tissue material must be included in the test.

Susceptible species

- *Cyprinus carpio* and its hybrids (e.g. common carp x goldfish or common carp x crucian carp) have to be collected when present

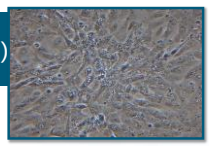
Molecular techniques for disease diagnosis

- Real-time PCR (Gilad Taqman assay, Gilad et al.,2004) shall be used - most sensitive and specific test & minimizes the risk of cross-contamination. [Details of primer & probe sequences are given]
- Alternatively the conventional PCR assay described by Bercovier et al. 2005 - targeting the TK gene of KHV - can be used. [Details of primer sequences are given]
- Conventional and real-time PCR assays with demonstrated similar sensitivities and specificities to the described assays may also be used.

Molecular techniques for surveillance

- Real-time PCR (Gilad Taqman assay, Gilad et al.,2004) shall be used - most sensitive and specific test & minimizes the risk of cross-contamination. [Details of primer & probe sequences are given]
- Real-time PCR assays with demonstrated similar sensitivities and specificities to the described assays may also be used.
- If positive samples appear in an area not previously confirmed positive - test results must be confirmed by sequencing of a PCR or nested PCR product (the generic nested PCR described in Engelsma et al. (2013) can be used - details of primer sequences are given) or sent to a reference laboratory for confirmation.
- Sequencing results can be analysed by aligning the sequences to the known reference sequences of KHV (Genbank accession numbers AP008984, DQ657948, DQ177346 (Aoki et al. 2007).

Cell culture isolation (cultivation)



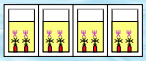
- Diagnosis of KHVD in clinically affected fish can be achieved by virus isolation in cell culture
- Cell culture isolation is not as sensitive as the published PCR-based methods to detect CyHV-3 DNA and is not considered to be a reliable diagnostic method for KHVD (OIE, 2013)
- Detailed procedures on cell cultivation are given in the OIE Aquatic Manual.

Other issues – definition of CyHV-3

- It was agreed that koi herpesvirus (KHV), which belongs to the family of *Alloherpesviridae* (Aoki et al. 2007, Waltzek et al. 2009) is the aetiological agent of KHVD.
- The scientific name is cyprinid herpesvirus 3 (CyHV-3), CyHV-3 isolates are defined as alloherpesviruses aligning 100% to the viral DNA polymerase gene and/or the major capsid protein gene of the CyHV-3 strains, KHV/J, KHV/U, and KHV/I, according to Aoki et al. 2007 (Genbank accession numbers AP008984, DQ657948, DQ177346, respectively).
- Therefore recently described novel strains of cyprinid herpesvirus (Engelsma et al. 2013) closely related to koi herpesvirus are not considered as CyHV-3, and thereby have not to be targeted by these surveillance procedures.

Serology

- Due to insufficient knowledge of the serological responses of fish to virus infections, the detection of fish antibodies to viruses has not thus far been accepted as a routine screening method for assessing the viral status of fish populations
- Validation of some serological techniques for certain fish virus infections could arise in the near future, rendering the use of fish serology more widely acceptable for health screening purposes **(OIE, 2013)**.



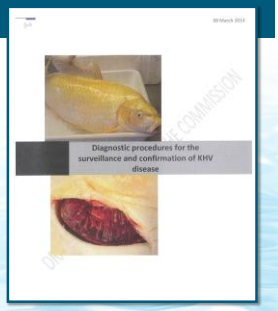
ELISA for antibody detection

Recommendation for further development

- The experts involved in this working group suggest that specific research activities are conducted to address **2 major issues** identified during the meeting:
  - **Serology procedures (e.g ELISA and seroneutralization)** – cross-reaction issues, kinetics of the antibody response in infected fish and the possibility for viral clearance in infected fish need further investigation
  - **Cyprinid herpes virus variants closely related to CyHV-3** - further studies are needed to elucidate if these variants have to be considered an emerging disease in Europe that may require a specific surveillance program

We hope that our recommendations to resolve these issues will be considered by the Standing Committee On the Food Chain and Animal Health (SCOFCAH).

Manual now available to download from the EURL Fish web site



That's all .....THANK YOU

'Fantastic carp' Land of a 1000 lakes in N.E. Bavaria