

UPDATE ON THE CEV SITUATION IN THE EU

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What is CEV (Carp Edema Virus) ?

- a poxvirus (E.M.) of carp and koi *Cyprinus carpio*
- first detected and described in Japan in the 70's (Murukami et al., 1976)
- severe viral edema of juvenile carp → high mortalities
- "Koi Sleepy Disease" (KSD) in older koi
- Older koi: lethargy: sleepy behaviour, fish lie on bottom and dies of anoxia
- losses in June to August in Japan, at temperatures 15 – 25°C
- mortalities up to 80%



Picture EUROL Fish Dis



History of CEV in Japan (H. Fukuda, pers. comm.)

- In Hiroshima and Niigata prefectures in **1970's**
- Development of 0.5% NaCl treatment
- Dissemination of NaCl treatment in **1980's**
- Identification of the agent CEV in **1990's**
- Finding of the same agent as sleeping disease in **2000**
- **Currently**, 0.5%NaCl treatment after grading is extensively conducted in koi and common carp industry (Miyazaki et al. 2005)
- It might make fish a virus carrier, which may be risky to unaffected fish throughout carp populations



Japan (H. Fukuda et al., pers.comm.)



Mass mortalities in carp & koi



Carp edema in small fish

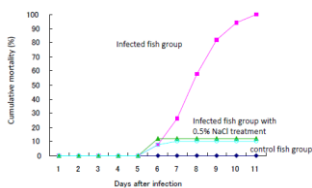


Koi Sleepy Disease in large fish

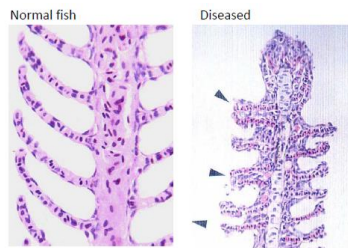


Japan (H. Fukuda et al., pers.comm.)

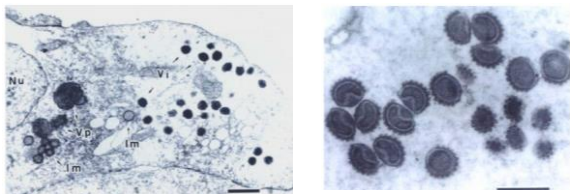
Effect of NaCl treatment on experimentally induced VEC



CEV histopathology: gills (Fukuda et al., pers.comm.)



T.E.M. of CEV : pox virus (Fukuda et al., pers. comm.)



History of CEV in **koi** in Europe



Picture EURL Fish Dis

- **UK:**
  - In 2009 CEV first detection in Europe, in **England** in imported diseased koi (again in 2011-2013 in diseased koi from hobby ponds) (Way & Stone, 2014)
  - 2013: Low levels of CEV-like virus in healthy koi imports from Israel and Japan at ornamental fish wholesalers in the **UK** (Way & Stone, 2014)
- **Germany:**
  - 2010 in imported koi from Japan (Bergmann, pers.comm.)
  - Spring 2014 (Jung-Schroers et al., 2015)



History of CEV in **koi** in Europe

- **France**, 2013 in imported koi (Bigarré et al., unpublished; Way & Stone, 2014)
- **Netherlands**, Sept 2013 in Japanese koi (Haenen et al., 2014)
- **Czech Republic**: koi since 2014 (Vesely, pers.comm.),
- **Austria**: since April 2014 (Lewisch et al., 2014)
- **Italy**: since 2014 (A.Toffan, M. Abbadi and V.Panzarin: pers.comm.)
- **Belgium**: in 2012, in koi from Japan
- other countries...?



2013: Clinics of 1st CEV detection in NL, in Japanese koi



Slight gill necrosis, slight exophthalmus, anorexia, haemorrhaged fins Internally: kidney granulomatous Pictures O.Haenen



2013: Clinics of 1st CEV detection in NL, in Japanese koi

- Frozen koi
- Enophthalmus
- Gill necrosis
- Anorexia

...post mortal & -freezing?



Pictures O.Haenen



CEV tests and results

- Cefas: modified nested PCR assay for CEV: detection & confirmation of CEV in imported and hobby koi with KSD signs
- Phylogenetic analysis (Cefas, poster K. Way et al.) of the amplification products ➔ 2 main lineages of CEV-like virus UK carp:
  - lineage 1: samples obtained from **koi: CEV**
  - lineage 2: samples from common **carp: CEV-like**

Remark: Within lineage 1, detections more similar to the original Japanese CEV distinguishable from the other detections from koi

- CVI developed a Sybr green qPCR based on sequences received from Cefas, now in use
- **TaqMan RT-PCR** developed at CEFAS (D.Stone & K. Way): chosen as standard test method



### CEV in carp

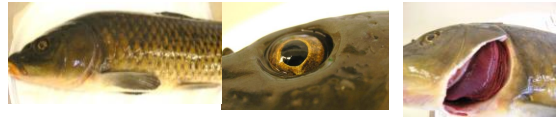
- In **2012**, CEV-like virus detected for the first time in **UK** in common carp showing KSD-signs (poster **K. Way et al.**) during periods of low water temperatures (**6-9°C**) in winter and early spring (Way & Stone, 2014).
- **March 2014** in **NL**: big carp: high mortality: 1 carp, no clinics, CEV positive, Ct value 28, water temp appr. **6°C**.
- **NL**: Did they die from this virus or was mortality due to the very soft winter??? Exotic birds botulism positive, carp negative.

Pictures ackn. carp board, NL



### CEV in carp

- **Mid Sept 2014** (end of summer): 450 carps died in 1 week
- Temp **18°C**
- 1 carp → CVI with lethargy, anorexia, enophthalmus, slight gill necrosis
- CEV positive, and KHV negative, but
- Still no proof of CEV as cause!



Pictures CVI,NL

### CEV in carp in Europe

- **Netherlands**, since April 2004 (Haenen et al.)
- **Austria** since March 2013 (Lewisch et al., 2014)
- **Czech Republic**, in carp since 2013 (Veselý, pers.comm.),
- **France**, 2010?
- **Italy** 2010 (+ CyHV-1)(A.Toffan, M. Abbadi and V.Panzarin: pers.comm.)
- **Poland**: autumn 2014: (Reichert et al., pers.comm.)
- Other countries?



### Double infections of CEV in NL

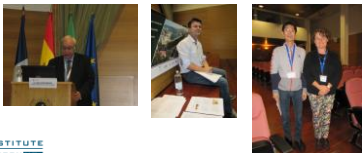
- **2004** : with **SVCV**
- **2011**: with **picorna-like virus**
- **2015 a.o.**: with **CyHV-1**
- ...Was CEV really causing disease in these cases?



Pictures CVI, NL

### Future: CEV network & proposal

- **Malaga Oct 2014: CEV network started, funding needed for:**
  - To develop SOPs for sampling, detection and typing of CEV for use by the NRLs for Fish Diseases in Europe, and validate the tests
  - To establish a multiplex PCR assay for simultaneous detection of CyHV 1-3 and CEV
  - To exchange materials used in diagnostics
  - To assess the possible presence and spread of CEV in the partner countries
  - To assess the possible impact of CEV for cultured carp and koi and wild carp in Europe, with a draft research project proposal for a future call



### CEV workshop at EURL Fish, Copenhagen 12<sup>th</sup>- 13<sup>th</sup> January 2015

- organized because of the increasing number of diagnostic cases where CEV was detected in diseased cyprinids (both koi and common carp, *Cyprinus carpio*).
- aim of the workshop to share knowledge, diagnostic protocols and material among participants and evaluate different strategies on how to tackle this issue
- 20 participants from 11 countries (10 Eur. & 1 USA)
- **EURL Fish Diseases, CVI of Wageningen UR, FLI, Uni Vet Med Hannover, ANSES, VRI Brno, Uni Vet Med Vienna, Uni S-Bohemia, CEFAS, Piwet Poland, NVI Oslo, IZSVE Italy, and Uni of Florida**



Picture EURL Fish Dis

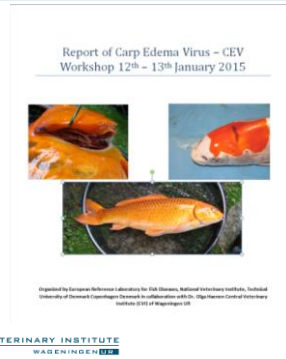
CEV workshop at EURL Fish, Copenhagen

organizers: Niccoló, Olga, Susie, Niels Jørgen



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a Report has been made of this workshop



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Outcome of workshop

- Strong network
- TaqMan RT-PCR developed at CEFAS which distinguishes koi CEV and carp CEV-like virus is available as test method (D.Stone & K.Way)
- Uni Hannover has developed a qPCR (M.Adamek et al.), which is good for koi CEV, and FLI has a nested PCR, as well fine (S.Bergmann et al.).
- Surveillance method needed to test if CEV is emerging in Europe and would be a candidate to be listed
- Water temps at CEV outbreaks: carp mostly at 6-9°C, koi mostly at 15-25°C
- Look into archive samples... Epidemiology
- Various CEV papers in preparation (K.Way et al. alert paper CEV in Europe)
- Novel viruses of cyprinids workshop EAFP Las Palmas: (Haenen, Waltzek and Way)
- Look into funding options for network & research CEV

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Conclusions

- CEV is present in Europe, since 2004:
  - CEV in (often big) koi since 2008/2009
  - CEV in (often big) carp since 2004
- Mortalities may be high in combination with stress/anorexia...
- Different PCR tests developed
- Not clear if CEV is emerging, as labs start CEV testing
- Impact of CEV infections for carp populations in Europe still unclear, Postulates of Koch?
- Alert paper *J. Fish Dis.* 2015 in prep (K.Way et al.)
- Surveillance and risk analysis on CEV in koi and carp, *Cyprinus carpio*, needs immediate attention
- Funding searched for networking and research

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Thank you, questions?

