

CARP EDEMA VIRUS DISEASE (CEVD) IN EUROPE – AN UPDATE

Olga Haenen (based on alert paper: The emergence of carp edema virus (CEV) and its significance to European common carp and koi, *Cyprinus carpio*: K. Way^{1*}, O. Haenen², D. Stone¹, M. Adamek³, S.M. Bergmann⁴, L. Bigarré⁵, N. Diserens⁶, M. El-Matbouli⁷, M.C. Gjessing⁸, V. Jung-Schroers⁹, E. Leguay⁹, M. Matras¹⁰, N.J. Olesen¹¹, V. Panzarin¹², V. Pláčková¹³, A. Toffan¹², N. Vendramin¹⁴, T. Veselý¹⁴, T. Waltzek¹⁵, 2017 resubmitted DAO)



Set up

- What is CEV, and what is KSD?
- The network
- New detections of CEV in Europe (since May 2016, 20th NRL meeting)
- News on diagnostic methods
- Genetic characterization of CEV strains
- New data from research papers
- Discussion & Conclusions



What is CEV/KSD?

- 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
- USA: since 1996 (Hedrick et al. 1997)
- Not notifiable for EU or OIE
- Transmission: horizontal, vertical?



Picture EURL Fish Dis



What is CEV/KSD?

- **Japan: Therapy:** 0.5%NaCl treatment after grading extensively conducted in koi and common carp industry (Miyazaki et al. 2005) ⇒ possibly fish becomes virus carrier
- **Europe: Water temperature** at CEV outbreaks:
 - carp mostly at 6-10°C (but also 15-25°C)
 - koi mostly at 20-23°C (but also at 9°C)



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



Mass mortalities in carp & koi

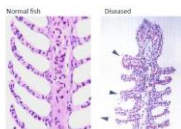


Carp edema in small fish

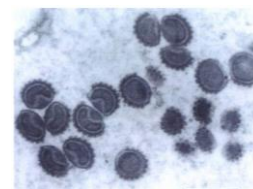
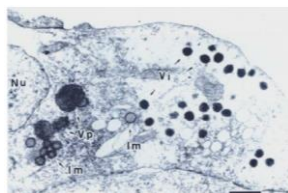


Koi Sleepy Disease in large fish

Hypertrophy and severe hyperplasia of branchial epithelial cells resulting in fusion of adjacent secondary lamellae and clubbing of the gill filaments (Ono et al. 1986; Miyazaki et al. 2005)



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



size 333-400 x 400-413 nm



History of CEV in Europe



Picture EURL Fish Diseases

KOI:

- **UK:** in **2011** CEV first detection in Europe, (**2009** 1st detection in Europe in Belgian koi: Way & Stone, 2014)
- **Germany:** since **2009**
- **Netherlands,** since 2013
- **Austria,** since 2014
- **Czech Republic,** 2014
- **France,** 2013
- **Italy,** 2014
- **Poland,** 2013
- **Switzerland,** **2007** (Wahli, pers. comm.)



History: CEV detection in **carp** in Europe:

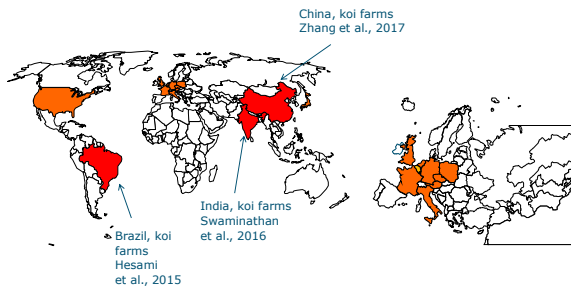
low # outbreaks, mortality may be high, sometimes + CyHV-1/CyHV-3/SVCV/other virus

- **UK,** detected in archive samples from 2004, **1999** and **1998.**
- **Germany,** since 2015
- **Netherlands,** since **2004**
- **Austria** since 2014
- **Czech Republic,** 2013
- **France,** 2010
- **Italy** 2010
- **Poland,** 2013
- Other countries?



CEV, May 2017 (K. Way et al., 2017)

new = red



The network: CEV workshop at EURL Fish, Copenhagen Jan 2015, and CEV discussion lunch meeting at EAFF Conference

Sept 2015 organizers: EURL Fish Diseases and Olga Haenen



Reports at EURL Fish Diseases website

Report of Carp Edema Virus - CEV
Workshop 12th - 13th January 2015

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Report of Carp Edema Virus -
CEV Discussion EAFF Conference,
Las Palmas,
lunch 9th September 2015

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CEV: New detections in Europe since May 2016

- **Poland** (NRL): as part of a KHVD surveillance programme, tissue samples from 36 carp farms between **2013 and 2015**. Archived samples later screened by real-time qPCR, 16 of the farms (**45%**) tested positive for CEV (**Matras et al. 2016**)
- **Italy** (NRL): Numerous outbreaks in 2016 (archive mat. since **2010**)
- **France** (NRL): in 2016 ≥ 2 KSD cases in common carp; owner treated the fish with salt and vitamin C \Rightarrow signs disappeared... (**L. Louboutin**)
- **UK** (CEFAS): in April 2016 1x detected in carp
- **Germany:** Numerous linked and un-linked cases
- **Czech Rep** (NRL): 2016: further positives in koi/carp
- **NL:** Aug 2016: 1x koi
- **Switzerland:** 2x koi in 2016; paraffin embedded gill tissue since 2007



CEV: News on diagnostic methods

- Japan: Oyamatsu et al., 1997 conv. PCR primers
- T. Miyazaki : unpublished sequence, CEFAS: P4a gene sequence ➔
- Cefas: modified real-time qPCR assay for CEV: (Way & Stone, 2013), also for formalin fixed paraffin material up to 15 years back positive (primers in this paper)
- High levels of CEV genome detected in **gill** tissue (Swaminathan et al. 2016)



Attempts to isolate the virus:

- Indian laboratory attempted to isolate CEV in many cell lines from different fish species ... **none** susceptible to the virus (Oyamatsu et al. 1997a, Swaminathan et al. 2016).
- Primary, explanted, cultures of gill tissue enabled replication of CEV, during 2 days, measured by mRNA expression. CEV replication was not detected in fin tissue (Adamek et al. 2017)
- ➔ Development of a **cell line** derived from **carp gill** epithelial tissue may provide the best opportunity to culture CEV *in vitro*



Genetic characterization of CEV strains

- France (Bigarré): two lineages partially sequenced (of 12 cases since 2013: ≥ 2 genotypes (maybe 3 depending of how we consider the clusters), common carp/koi differ
- Uni Vienna did sequence work ➔ 2 lineages
- PIWET Poland: Matras et al. 2016 (in cooperation with Stone and Way) *J. Fish Dis.* 2016 on **3rd lineage**
- Phylogenetic analysis (Cefas) ➔ 3 main lineages of CEV:
 - lineage 1: samples obtained from **koi/carp**
 - lineage 2: samples from common **carp**
 - Lineage 3: samples from **Polish common carp** (links with import of koi from Asia)



CEV: News from infection experiments

- Experimental virus transmission studies, **gill tissue** from CEV-infected common carp had significant higher virus loads than kidney, spleen, skin and gut (Adamek et al. 2017)
- CEV disease affected **koi**: a mean of 129,982 copies of CEV-specific DNA per 250ng of extracted DNA (Adamek et al. 2016)
- CEV disease affected **common carp**: a mean of 504,000 copies of CEV-specific DNA per 250ng of extracted DNA (Adamek et al. 2017)
- Common carp, naturally infected with genogroup I CEV and koi, naturally infected with genogroup II CEV, transmitted the virus to naïve common carp and koi (Adamek et al. 2017)
- Strains of common carp showed varying levels of resistance to CEV infection: The Amur wild carp strain was resistant to both genogroup I and II CEV and did not develop clinical CEVD (Adamek et al. 2017).



Management and prevention (Hesami et al., 2015)

- **Good health management practices** should be followed, quarantining and testing new arrivals, avoiding crowding and stressful events, maintaining good water quality and providing good quality nutrition
- **Quarantine** requirement is minimal 30 days at 15 - 25°C, in closed facilities to avoid CEV contamination from wild or feral carp
- **Avoid overstocking**, in open waters, including wild/feral carp.
- If a disease outbreak is suspected, immediately **remove diseased fish**, to minimize disease transmission in the population



Management and prevention (Hesami et al., 2015)

- Fish returning from a **koi show**: place in quarantine before return to the general population. Also, as it is not known whether survivors of CEVD outbreaks become lifelong viral carriers, it is recommended that survivors are not taken to koi shows or cohabitated with naïve carp/koi
- Without sensitive and reliable diagnostic methods, to detect sub-clinical carrier fish, absence of the disease cannot be ascertained and therefore disease-free areas cannot be documented. Then **legislation is of little use** to control and prevent incursion of a virus disease
- Based on the above, and also the difficulty in fulfilling Koch's and River's postulates for CEV ➔ **small chance** that CEVD would be considered for **listing** as a notifiable disease



Knowledge gaps

- CEV may be a significant disease agent in previously unexplained carp mortalities in spring. In England, **spring carp mortality syndrome (SCMS)** was first reported in the 1980's and first investigated by the Environment Agency and Cefas in the late 1990's.
- Persistence of the virus in the aquatic environment, risk of recurrence of infection and the possible trigger events that promote disease outbreaks?
- Stress-related reactivation of a persistent CEV infection, like suspected in fowl pox infections? **Adamek et al., 2016**
- Transmission of CEV and the vectors involved?
- Improve virus purification methods and establish cell lines... develop diagnostic methods, study pathogenicity, to establish Koch's postulates and investigate the antibody response in carp



Persistence of CEV

Adamek et al. (2017):

- CEV not confirmed by qPCR in fish surviving clinical CEVD, one month after the last clinical signs.
- These fish did not transmit CEV to naïve fish.
- Suggest, that survivors of CEVD clear the virus and do not develop a persistent sub-clinical infection.
- However, it is suggested to explore the influence of stress in carp that survived CEVD



Recommendations

- Add PCR-based assays to national fish health monitoring surveillance programs in the EU, after validation for use for koi and carp
- CEV causes immunosuppression, resulting in the establishment of extensive secondary bacterial and parasite infections. Analysis of blood parameters in affected fish to confirm immune impairment? Koch's or River's postulates? to confirm that CEV is the primary causal agent of the disease with which it is associated?
- Develop antibody-detection assays as an important tool for disease surveillance
- Study the disease epidemiology, pathogenesis and immunogenesis to assess of the impact of the disease in Europe. ... **Research funding is needed!**



Conclusions

- Some new CEV detections in Europe, and new in India, China, Brazil
- Link with SCMS in UK since 1998
- Lack of susceptible gill cell lines hampers further research, *in vitro* & *in vivo* studies, like Postulates of Koch trials, etc.
- Role of CEV? Emerging? Still unclear. Many knowledge gaps
- Funding** needed for research
- CEV Network is active, please inform me



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Thank you for your attention