

## CARP EDEMA VIRUS IN EUROPE, CURRENT STATUS

Olga Haenen<sup>1</sup>, Keith Way<sup>2</sup>, Niccolò Vendramin<sup>3</sup>, David Stone<sup>2</sup>, Michal Reichert<sup>4</sup>, Marek Matras<sup>4</sup>, Charlotte Axén<sup>5</sup>, Sven Bergmann<sup>6</sup>, Laurent Bigarré<sup>7</sup>, Lénail Louboutin<sup>7</sup>, Mikolaj Adamek<sup>8</sup>, Verena Jung-Schroers<sup>8</sup>, Tomáš Veselý<sup>9</sup>, Bartolomeo Gorgoglione<sup>10</sup>, Oskar Schachner<sup>11</sup>, Mansour El Mattboul<sup>10</sup>, Veronika Pláčková<sup>12</sup>, Valentina Panzarin<sup>13</sup>, Anna Toffan<sup>13</sup>, Miriam Abbadi<sup>13</sup>, Vlasta Jencic<sup>14</sup>, Susie Sommer Mikkelsen<sup>15</sup>, Niels Jørgen Olesen<sup>3</sup>



CENTRAL VETERINARY INSTITUTE  
 WAGENINGEN

## Set up

- What is CEV, and what is KSD?
- The network
- New detections of CEV in Europe
- Isolation and replication of CEV: what has been done?
- Diagnostic methods
- Genetic characterization of CEV strains
- New infection experiments
- Discussion & Conclusions

CENTRAL VETERINARY INSTITUTE  
 WAGENINGEN

## 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

CENTRAL VETERINARY INSTITUTE  
 WAGENINGEN

## 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 6-9°C (but also 15-20°C)
  - koi mostly at 16-23°C (but also at 9°C)

CENTRAL VETERINARY INSTITUTE  
 WAGENINGEN

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



Mass mortalities in carp & koi

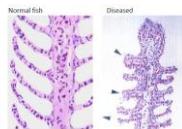


Carp edema in small fish



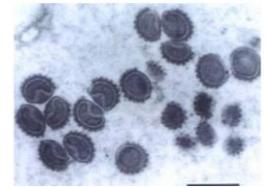
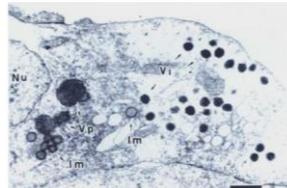
Koi Sleepy Disease in large fish

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)



CENTRAL VETERINARY INSTITUTE  
 WAGENINGEN

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



size 333-400 x 400-413 nm

CENTRAL VETERINARY INSTITUTE  
 WAGENINGEN

### History of CEV in Europe



**KOI:**

Picture EURL Fish Diseases

- UK: in **2011** CEV first detection in Europe, (2009 1<sup>st</sup> 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
- Other countries?



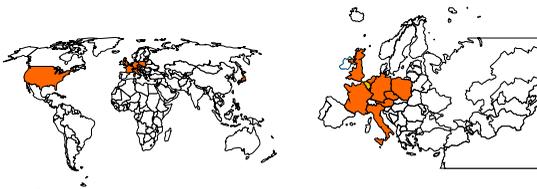
### History: CEV detection in **CARP** in Europe:

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

- UK, since 2004
- Germany, since 2015
- Netherlands, since 2004
- Austria since 2014
- Czech Republic, 2013
- France, 2010
- Italy 2010
- Poland, 2013
- Other countries?
- **Alert paper *J.Fish Dis.* 2016 in prep (K.Way et al.)**



### CEV, where, May 2016?



...Not many countries, but....which countries screen/test for CEV?



### .... Concern about CEV in EU and OIE...



...CEV might harm carp culture, koi culture and -trade, and wild carp populations:

**Is CEV emerging and a candidate for listing?**



### CEV workshop at EURL Fish, Copenhagen Jan 2015, and CEV discussion lunch meeting at EAFF Conference Sept 2015

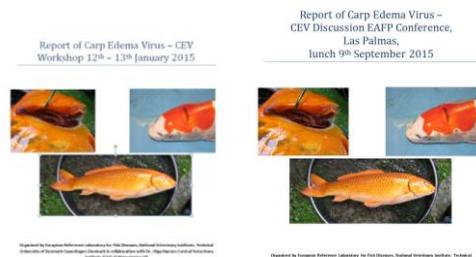
organizers: EURL Fish Diseases and Olga Haenen



<http://www.eurl-fish.eu/Reports>

### Reports at EURL Fish Diseases website

Outcomes: please see abstract of this lecture



<http://www.eurl-fish.eu/Reports>

### CEV: New detections in Europe

- Poland (NRL):** since Jan 2015: monitoring program: 23 (2x koi) of 54 (2x koi) samples positive :
  - 22x no clinics
  - 1x koi 90% mortality with lethargy and pale necrotic patches on the gills
- Italy (NRL):** since Jan 2015 4 new CEV cases: 2 koi (2015), 2 carp (2016)
- France (NRL):** in 2016  $\geq$  2 KSD cases in common carp; owner treated the fish with salt and vitamin C  $\rightarrow$  signs disappeared
- UK (CEFAS):** in June 2015, 1 detection in carp, in April 2016 1 detection in carp



### CEV: New detections in Europe

- Germany (Uni Hannover):** Samples from whole country (with/no clinics) in 2015 tested (and questionnaire being analysed): total 40,5% CEV positive

Species	No. of samples examined	No. of positive tested samples
Koi	179	62
Common Carp	73	40
In total	252	102

- Czech Rep (NRL):** 2015: 2 or 3 PCR positives (common carp); 2016: 2 positives, several suspicions (pending diagnosis)
- NL:** in 2015, 3x carp; April 2016, 1x big carps, high mortality. Ct=30.
- Other countries?.....???**



### CEV: Diagnostic methods, examples:

- Japan:** Oyamatsu et al., 1997 conv. PCR primers
- T. Miyazaki :** unpublished sequence, CEFAS: P4a gene sequence  $\rightarrow$
- Cefas:** modified real-time qPCR assay for CEV: (Way & Stone, 2013), also for formalin fixed paraffin material
- Uni Hannover:** qPCR (Adamek et al.)
- CVI Lelystad:** experimental SybrGreen qPCR (Engelsma)
- ANSES:** qPCR CEFAS validated (M. Baud)
- Other?**



### Virus isolations: Cells tested by (co)authors:

no cpe so far

Cell	Tested? at temp?	Remark: tested by various labs
EPC, BF-2, FHM	Yes, at 15,20 & 26°C	
RTG-2, trout & 7 other salm. cells, like CHSE-214, SSE	Yes, at 15,20 & 26°C	
CCB, carp	Yes, 15, 20 & 26°C	Up to 10 subcultivations (Cz.Rep)
KF-1, koi fin	Yes, at least 20°C	
7 other cyprinid cells, 4 eel cells, 9 other fish cells	Yes, 20°C	(Japan: Oyamatsu)

Based on data from CEFAS, Uni Vienna, ANSES, Uni Hannover, VRL Czech Republic, CVI NL



### Virus isolations: Cells tested : no cpe so far

Cell	Tested? at temp?	Remark
CCF (M. Imajoh, Japan, ackn.)	Not yet	Uni Vienna (Gorgoglione et al.)
4 days old fin primary cells carp	25°C	Uni Hannover (Adamek et al.) : Traces of viral mRNA - not suitable for CEV replication
Fresh carp gill explant cultures	15°C	Uni Hannover: Viral mRNA detected - virus replicates.
Fresh carp gill explant cultures	25°C	Uni Hannover: Viral mRNA detected - virus replicates $\rightarrow$ need the cell line raised from gill (likely epithelium)
SHK, WSSK, SBL, SSN1, PA4, LEB, a.o.	14°C	ANSES (M. Baud et al.) with gills from koi experiments
Duck Fibroblast (DF-1), Lamb Kidney (LN2), primary Chicken Embryo Fibroblast (CEF)	20°C, 24°C	CVI, NL (Haenen et al.): 5x koi and 3x carp samples
NGF (T. Ito, Japan, ackn.) Koi (fibroblast)	Yes, at 15,20 & 26°C	CVI, NL (Haenen et al.)

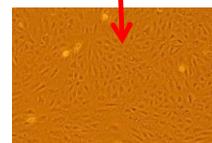
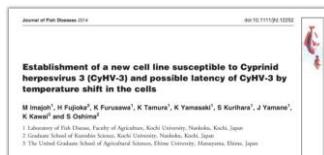
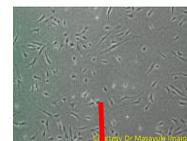


### Austria: CCF-K104 cell line:



New cyprinid cell line from Japan  
Kindly provided by Dr Masayuki Imajoh from Kochi University

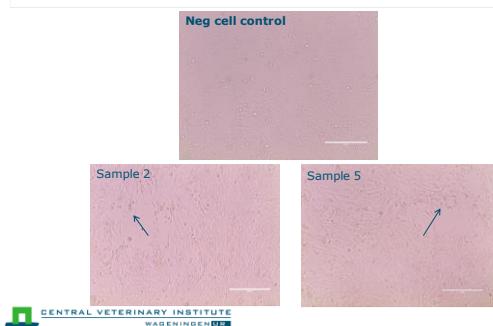
Fibroblastic cells from caudal fin of adult common carp  
Recommended for isolation and productive replication of CyHV-3



CCF at arrival on 23 Feb 2016



DF-1 Duck Fibroblast cells : 3 dpi at 24°C  
 ...cpe? → no increase of virus titre



CENTRAL VETERINARY INSTITUTE  
 WAGENINGEN

### 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: publication Matras et al. 2016 (in cooperation with Stone and Way) accepted *J. Fish Dis.* on 3<sup>rd</sup> 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 comm** with import of koi from Asia)

CENTRAL VETERINARY INSTITUTE  
 WAGENINGEN

File Name: DF-1 Duck fibroblast cells (20160616) (1).indd 1  
 Author: J. Bigarré, D. Stone & M. Way (2016) (16/06/2016)  
 Location: C:\Users\J. Bigarré\Desktop\Publications\2016\20160616 DF-1 Duck fibroblast cells (20160616) (1).indd 1  
 Application: Microsoft PowerPoint 2013  
 Date: 16/06/2016 10:00:00  
 The application (Microsoft PowerPoint 2013) was last used on 16/06/2016 10:00:00.  
 © 2016 Microsoft Corporation. All rights reserved. Microsoft PowerPoint 2013 and 2013 are either registered trademarks or trademarks of Microsoft Corporation in the United States and/or other countries.

### CEV: New infection experiments

- Germany, FLI: Experiment from a natural KSD outbreak in "Thuringia" koi where other cyprinids showed the same clinical signs and mortality as koi.
- Poland, NRL: transmission of CEV to other species by co-habitation, results not conclusive yet
- Austria, Uni Vienna: B. Gorgoglione et al., see next slide
- France, NRL: movie: <http://mesechanges.anses.fr/htcomnet/Handlers/AnonymousDownload.ashx?file=436352e3>
- Czech Rep, NRL: One unsuccessful trial to infect naive carp with gill homogenate

CENTRAL VETERINARY INSTITUTE  
 WAGENINGEN

**Transmission trials:**

Sick koi co-habited with healthy common carp  
 Insurgence of typical clinical signs within 6 h  
 Viremia already detectable at 24 h p.e.  
 Viremia reaching the peak at 7 days p.e.  
 Carp dead at 7 days p.e., virus detectable also from internal organs

Other carp and koi survived, showing constant mucus positivity even after several months

CENTRAL VETERINARY INSTITUTE  
 WAGENINGEN

Amplifcations from the second step of nested PCR (CEFAS, 2013 protocol)

Haenen O., Way K., Gorgoglione B., Ito T., Paley R., Bigarre L., Waltzek T. (2016). Novel viral infections threatening Cyprinid fish. *Bull. Eur.Ass.Fish Pathol.* 36(1) 11- 23.

## Carp Edema Virus : Experiments performed at ANSES (Unit Viral Diseases of Fish – French Ref Lab) in 2015

Joëlle CABON, Lénaig LOUBOUTIN, Marine BAUD  
 & Thierry MORIN

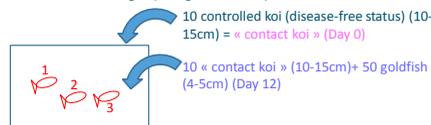


V15/3 (25/04/2015) : information on farm conditions : water temperature 14°C

3 koi with clinical signs (letargic ; 10-15cm)

DATA ANSES:

Non-regulated water temperature, mean temperature = 13°C



Day 0 : koi 1 ♀ : PCR\* CEV + (gills, Ct=20)  
 Day 1 : koi 2 ♀ : PCR CEV + (gills, Ct)=26)  
 Day 2 : koi 3 ♀ : PCR CEV + (gills, Ct)=22)

Appearance of typical clinical signs (see short movie)

Day 10 : ♀ of a first « contact koi » : PCR CEV +

Day 12 : addition of 10 other controlled koi and 50 goldfish

Day 12 : ♀ of a second « contact koi » : PCR CEV +

Day 16 : ♀ of a third « contact koi »

Day 23 (11 days after second addition of controlled koi) : ♀ 1 koi

CENTRAL VETERINARY INSTITUTE  
 WAGENINGEN  
 \* : real time PCR, according CEFAS's method

**V15/3 (25/04/2015)**



**DATA ANSES:**

Non-regulated water temperature, mean temperature = 13°C

Surveillance of mortality during 4 months : no more death

**Conclusion :** 4 « contact koi » 20 ; no 20 for goldfish

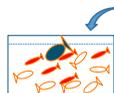
⇒ PCR performed on survivors : pools of 2 koi and 5 goldfish  
 Contact koi : 2/2 PCR+ (late C(t) 36-37, detected or not regarding sampling (internal organs, gills, or muscles)  
 Contact koi : 1/2 PCR+  
 Goldfish : 2/4 at late C(t) (36-37)



**V15/6 (09/07/2015) : information on farm conditions : water temperature 22°C, 100% mortality within one month**

**DATA ANSES:**

09/07: 33 koi with clinical signs (letargic ; 10-15cm)  
 12/07: 16 dead koi ; 2 koi tested PCR CEV + (C(t)=26)  
 15/07: 13 dead koi  
 16/07 : 3 dead koi



Water temperature = 17°C-18°C

16/07 (Day0) : transfer of one survivor with 76 controlled koi (disease-free status) and goldfish (8-10cm)

Koi mortality	
day1	
day2	
day3	Clinical signs only on koi
day4	9
day5	6
day6	15
day7	12
day8	12
day9	5
day10	5
day11	5
day12	1
day15	1
day18	1
total	71/76

**Conclusion :** high mortality on koi ; water temperature higher than for the previous experiment



**V15/6 (09/07/2015)**

**DATA ANSES:**



**Conclusion :** no mortality on koi and goldfish after one month  
 ⇒ Goldfish would not be vector for CEV?

Remark : only isolates from koi have been used for those experiments



CEV new proposals?

- Uni Vienna: B.Gorgoglione: 5 kC CEV virus isolation project
- CEFAS: one study on characterization of CEV by genome sequencing as part of a Defra project
- GillGutSkinNET COST Action (Frode Paulsen, NVI Oslo) submitted
- Other projects???
- Any calls foreseen to apply for?



Conclusions

- Role of CEV? Emerging? Still unclear. Some new outbreaks in Europe
- 3 lineages of CEV exist
- Lack of susceptible cells hampers further research, *in vitro* & *in vivo* studies, like Postulates of Koch trials, etc.
- Alert paper Way et al. almost ready to submit *J. Fish Dis.*
- Funding needed for networking, virus isolation, other research
- CEV Network is active, please contact Olga to be added



Thank you



We thank our colleagues from the various labs, who did the work for this presentation:

France: ANSES: Marine Baud and Laurane Pallandre, Emmanuel Legay (privete vet) Joëlle Cabon, Thierry Morin  
 Netherlands, CVI: M. Voorbergen, B. van Gelderen,  
 I.Roozenburg, K.Weerdmeester, A. Dekker, M. Engelsma  
 .....and colleagues from the other labs

Bull. Eur. Ass. Fish Pathol., No(1) 2016, 11

WORKSHOP

Novel viral infections threatening Cyprinid fish

O. Haenen<sup>1</sup>, K. Way<sup>2\*</sup>, B. Gorgoglione<sup>3</sup>, T. Ito<sup>4</sup>,  
 R. Paley<sup>5</sup>, L. Bigarre<sup>6</sup> and T. Waltzek<sup>6</sup>

<sup>1</sup>Central Veterinary Institute of Wageningen UR, The Netherlands; <sup>2</sup>Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Wymouth DT99 9UB, England; <sup>3</sup>Clinical Division of Fish Medicine, University of Veterinary Medicine, Universityplatz, 1, 1210 Vienna, Austria; <sup>4</sup>Zemlin Laboratory, Aquatic Animal Health Division, National Research Institute of Aquaculture (NRIAS), Fisheries Research



[https://www.researchgate.net/publication/297460734\\_Novel\\_viral\\_infections\\_threatening\\_Cyprinid\\_fish](https://www.researchgate.net/publication/297460734_Novel_viral_infections_threatening_Cyprinid_fish)