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## FIRST CASE OF KOI SLEEPY DISEASE (KSD) BY CARP EDEMA VIRUS (CEV) IN THE NETHERLANDS

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Haenen O.<sup>1</sup>, Way K.<sup>2</sup>, Stone D.<sup>2</sup> and Engelsma M.<sup>1</sup>

<sup>1</sup>CVI, Lelystad, NL; <sup>2</sup> CEFAS, Weymouth, UK



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## What is CEV (Carp Edema Virus) ?

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- 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 oedema 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 spring and autumn in Japan, at temperatures 15 – 25°C
- mortalities up to 80%

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## History of CEV in koi in Europe

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- In 2009 CEV first detection in Europe, in England in imported diseased koi (again in 2011-2013 in diseased koi from hobby ponds)
- Low levels of CEV-like virus detected in healthy koi imports from Israel and Japan at ornamental fish wholesalers during 2013 in the UK (Way & Stone, 2014)
- in Germany in imported koi from Japan in 2008 (Bergmann, unpublished)
- in France (Bigarré et al., unpublished; Way & Stone, 2014) in 2013 in imported koi
- In 2013 in Netherlands (this lecture)

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## CEV tests and results

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

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

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## Casus in diseased koi in NL

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- Sept 2013: High mortality in young and older own koi (1 year ago imported from Japan)
- 2 months after adding 6 koi from Japan: 2 "survivors"
- kept at 20°C, own koi showed "sleepy" behaviour
- Treated with commercial antiparasitic and antibiotic
  - ➔ no effect
- CVI: apathy, anoxia, anorexia, enophthalmus, gill necrosis with oedema, slight anemia, and many *Gyrodactylus* spp. in the gills
- Internally, no abnormalities
- Suspicion of KSD ➔ double sampling, 1 set sent to Cefas
- 2 Japanese koi ➔ CVI ➔ Cefas

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## Clinics of 1st Japanese, CEV positive, live koi

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Slight gill necrosis, further slight exophthalmus, anorrexia, haemorrhages in fins

Internally: kidney granulomatous(?), histology due

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## Clinics (2nd, dead, frozen Japanese koi)

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- Enophthalmus
- Gill necrosis
- Anorrexia
- (post mortal & -freezing?)

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## Test results CEV in KOI

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- CVI: Casus: KHV test negative
- Cefas confirmed this first detection of CEV in koi by PCR: analysis ➔ very close similarity (99.5%) to the original Japanese CEV
- 2 Japanese koi : tested positive for CEV by Cefas and CVI
- Dutch koi archive samples since 1996 at CVI ➔ no positives yet

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## Follow up of casus

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- All Japanese koi, that were imported 1 year before died
- Owner flushed pond, put koi in quarantine, kept water temperature at 20°C and added salt (1 g/liter) ➔ the two new Japanese koi started feeding again. However, 1 died (put in freezer) and both ➔ CVI next day
- Further: Older koi (source not Japan according to owner) still alive at this site

(Japan: Miyazaki et al. 2005: 0.5% NaCl after grading koi prevents KSD outbreak)

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## CEV in carp?

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- In 2012, CEV-like virus detected for the first time in UK in common carp showing KSD-signs (from a cluster of fishery sites in south-east England and a site in the English Midlands) during periods of low water temperatures (6-9°C) in winter and early spring (Way & Stone, 2014).
- March 2014 in NL: carp 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.

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## CEV in Dutch archive samples of carp: 2x

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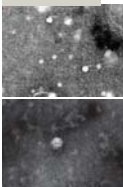
- Archive samples since 1993: CEV detected in diseased wild common carp in April: 2004 and 2011 (temp appr. 6-9°C normally)
- 2004 case: SVCV & CEV; 2011 case: picorna-like & CEV
- Was CEV really causing disease in these cases?

2004 casus, with SVCV



Carp had SVC signs

2011 casus



Small haem mouth, slight exophthalmus, many ectoparasites, congested kidney, pink muscle, haem testes, slimy viscera, no bacteria

T.E.M.: picorna-like, diameter 30 nm

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## Future

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### Cefas and CVI

- Try to culture the virus at various fish cells
- Do E.M.
- Histopathology

### Further:

- Serological tests?
- Pathogenesis studies? Etc.

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## Future: CoVetLab CEV proposal (2014-2015)

Network project NL, UK, France, Denmark, Sweden, Germany and Poland:

- To develop SOPs for sampling, detection and typing of CEV for use by the NRLs and regional laboratories for Fish Diseases in Europe.
- To establish a multiplex PCR assay for simultaneous detection of CyHV 1-3 and CEV
- To exchange materials used in diagnostics
- To validate the tests
- 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

This would benefit all labs testing for carp viruses

Proposal is under review



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## Conclusions

- CEV is present in Europe, at least since 2004:
  - CEV in koi since 2008/2009
  - CEV in carp since 2004
- Many questions on CEV
- The impact of CEV infections for carp populations in Europe is still unclear
- Therefore, surveillance and risk analysis on CEV in koi and carp, *Cyprinus carpio*, is important and should receive attention



Thank you for your attention!

Picture: city of Lille

