



Molecular tracing of viral pathogens in aquaculture

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NRL Fish Diseases
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Molecular tracing of koi herpesvirus (KHV) / Cyprinid herpesviruses 3 (CyHV-3)

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in cooperation with

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Cyprinid herpesviruses

Order: *Herpesvirales*

Family: *Alloherpesviridae* (Herpesviridae, Malacoherpesviridae)

Genera: Batrachiovirus Ictalurivirus Salmonivirus **Cyprinivirus**

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Species of Cyprinivirus

Cyprinid herpesvirus 1 (CyHV-1, carp pox virus) DD

Cyprinid herpesvirus 2 (CyHV-2, goldfish herpesvirus) DD

Cyprinid herpesvirus 3 (CyHV-3, koi herpesvirus)

Anguillid herpesvirus 1 (AnghV-1, eel herpesvirus)

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Cyprinid herpesvirus 1 (CyHV-1, carp pox virus)

„Carp pox“ or „Fish Pox“ or „*Epithelioma papillosum*“ or „*Carp Epithelioma*“

- skin disease of cyprinids (carps and minnows)
- milky-white to grey tumors
- juvenile fish with a high mortality
- lesions usually develop in low temperatures (winter/spring) and regress with high temperatures (summer) but the latent infection

(latency or persistence)

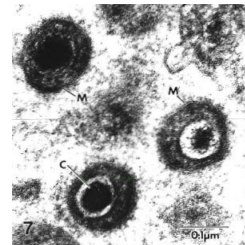
- The virus and the disease is present in most European countries.

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The agent carp pox virus


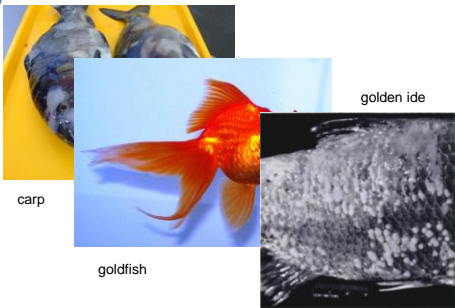
- ds DNA genome
- size 291,144 bp
- 143 ORFs (???) proteins
- obviously epitheliotropic
- pathogenesis is partly unknown



Steinhagen et al. 1992

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carp

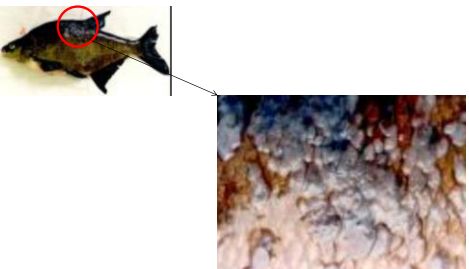
golden ide

goldfish


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Bream (*Abramis barma*, CEFAS 1994)



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Cyprinid herpesvirus 2

(CyHV-2, goldfish haematopoietic necrosis virus)

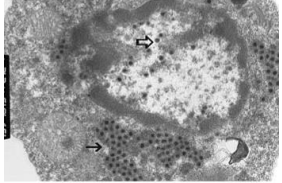
- latent infection in goldfish populations (stress = outbreak)
- anemia
- spring and fall, (warm water)
- small patches on gills and skin (rough areas)
- bleedings in different tissues
- necrosis in head kidney and kidney

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The agent goldfish herpesvirus (GHV)

- ds DNA genome
- size 290,304 bp
- 154 ORFs (??? proteins)
- enveloped
- target cells not known
- pathogenesis is partly unknown

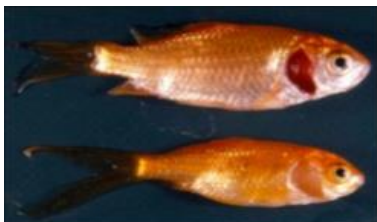


NMAHTVIG, Slide of the Quarter (April - June 2006) - Cyprinid herpesvirus 2
Australian Government

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The external examination of goldfish showed haemorrhages at different points of the body and fins, swollen anus, presence of haemorrhages in gills and eyes.




A goldfish with normal red gills (above) and a goldfish with anemia and the pale gills typical of GHV disease (below).

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CyHV-2 infection in crucian carp, *Carassius carassius* (Italy, UK)

Haemorrhages and bleedings



Fichi et al. 2103

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goldfish, *Carassius auratus auratus* (world-wide)

crucian carp, *Carassius carassius* (I, UK)

Prussian carp, *Carassius carassius gibelio* (China, G, CZ)

common carp, *Cyprinus carpio* (pers. comm., UK)



Summary on CyHV-2

1. GHV is a wide-spread herpesvirus in Europe.
2. GHV induces disease in several species.
3. GHV can cause latent or persistent infection.
4. Genomic investigation are hardly proceeded.
5. Only one virus genome is completely sequenced.
6. CyHV-2 was used for differential diagnosis.



Species of Cyprinivirus

Cyprinid herpesvirus 1 (CyHV-1, carp pox virus)

Cyprinid herpesvirus 2 (CyHV-2, goldfish herpesvirus)

Cyprinid herpesvirus 3 (CyHV-3, koi herpesvirus)

Anguillid herpesvirus 1 (AngHV-1, eel herpesvirus, HVA)



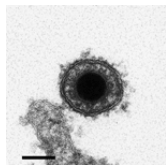
CyHV-3 (koi herpesvirus, KHV) (carp nephritis and gill necrosis virus, CNGV)

- KHVD is a disease caused by a highly contagious virus that causes significant morbidity and mortality in common carp and koi (*Cyprinus carpio*) varieties (Hedrick et al. 2000).
- only carp or koi are affected by the diseases
- at least 17 species are recognized serve as a virus replicating carrier (fish, mollusks, perhaps crustaceans)

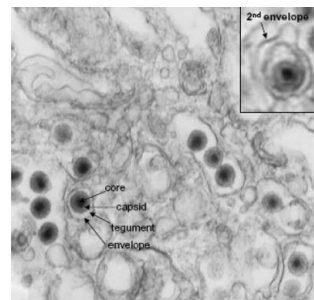


The agent CyHV-3 (KHV)

- ds DNA genome, 295 kbp (156 ORF - I, U, J)
- largest herpesviral genome
- 40 – 43 proteins
- major capsid protein CyHV-1 und -2
- four isolates are completely sequenced (2007-2014)
- in the Moltraq project: 19 KHV completely sequenced, 9 KHV partially



Mettenleiter et al. 2009



Hanson et al. 2011





Wild carp

farmed carp



KHVD in Indonesia

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Selected susceptible species for infection with KHV

- Goldfish** (*Carassius auratus*) (Bergmann et al. 2004, Sadler et al. 2007, El-Matbouli et al. 2007, Meyer 2007, Bergmann et al. 2010)
- Crucian carp** (*C. carassius*) (Kempster and Bergmann 2007, Bergmann et al. 2008, Bergmann et al. 2009)
- Grass carp** (*Ctenopharyngodon idella*) (Bergmann et al. 2009)
- Ide** (*Leuciscus idus*) (Bergmann et al. 2009)
- Silver carp** (*Aristichthys nobilis*) (Kempster and Bergmann 2007)
- Balck head carp**(*Hypophthalmichthys molitrix*) (Kempster and Bergmann 2007)
- Tench** (*Tinca tinca*) (Meyer 2007)
- Vimba** (*Vimba vimba*) (Kempster and Bergmann 2007)
- Hybrids crucian carp - koi** (Bergmann et al. 2010) KHVD
- Hybrids goldfish- koi** (Bergmann et al. 2010) KHVO

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Summary KHV (so far)

- KHVD only occurs in carp or koi
- KHV poses the largest herpesviral genome ("very old" virus)
- KHV is not species specific at all
- KHV is replicated to 50% without an envelope
- KHV is replicated in a lot of different fish
- KHV "development" is depending on water temperatures (warm water in Asia, cold water in Europe)
- out of a cloned virus up to 5 "different" viruses were identified by deep sequencing

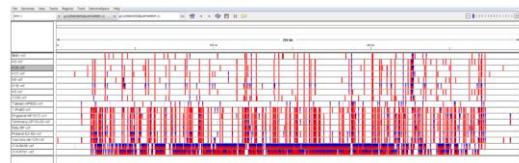
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Results from the project "Moltraq"



Sequence variation along the entire genomes



KHV-J as reference
 3 « groups »
 Confirms mixed infections or the development from one origin virus?
 white: there are no variations (to reference KHV-J)
 red: 100% of the specimen sequences differ from the reference one
 blue: specimen sequences are identical to the reference and another portion differ.

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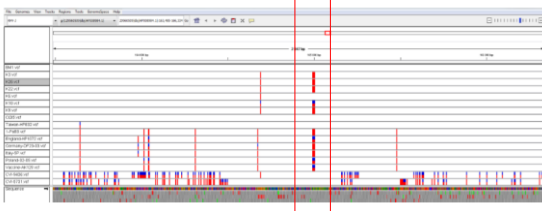


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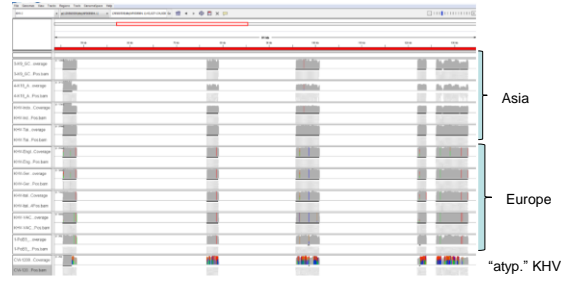
Enlargement of the diagnostic used region (Gilad et al. 2004)



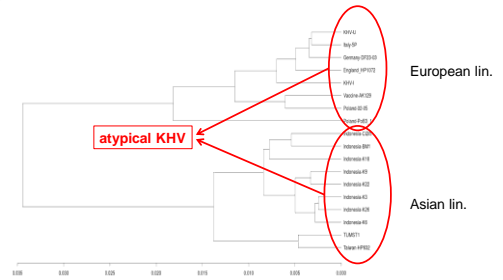
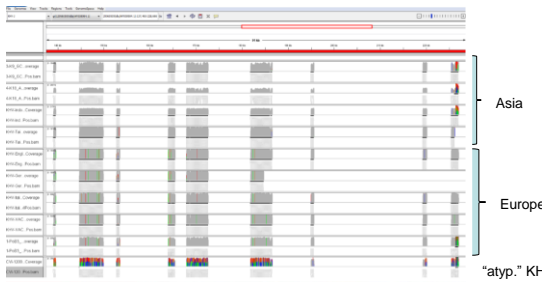
white: there are no variations (to reference KHV-J)
 red: 100% of the specimen sequences differ from the reference one
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Regions of interest only (1)



Regions of interest only (2)



What does that mean?



Virus cloud

One virus

Virus variations will infect the carp (cloud) *Where do they come from?*

One virus infects the carp
 Virus replication is different (depends on temp., host, organ etc.)

Several viruses are detectable but one is the major one (80-90%)

One virus will be the major (80 – 90%)
 - most virulent -

Other viruses are detectable

Other viruses are in as well



KHV so far:

1. All sequenced KHV are identical (99,98%)
2. One is a bit different (97% identity)

EU = DNA polymerase 98% identity?



What may help us?

- investigation of each single virus sequence from each single isolate (selected genes only)
- comparison with the consensus sequence of each isolate
- preparation of a map for genetic variation within one isolate
- with this help a map for genetic identification (Moltraq goal)

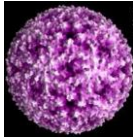


Thanks to all

- colleagues and friends from the Moltraq project (Saliha, Jean-Christophe, Michael, Heike)
- colleagues from CEFAS (Keith, David)
- colleagues from Italy (Valentina, Anna, Alice)



"A virus is a piece of bad news wrapped in a protein layer,"
Sir Peter Medawar



1960 [Nobel Prize in Physiology or Medicine](#) together with Sir [Frank Macfarlane Burnet](#).

