

CYPRINID HERPESVIRUS 2 (CyHV-2)

O. Haenen*¹, T. Ito², Duan Hongan³

¹ NRL, Wageningen Bioveterinary Research of Wageningen UR, Lelystad, the Netherlands

² National Research Institute of Aquaculture, Fisheries Research Agency, Mie, Japan

³ Lianyungang Entry-exit Inspection and Quarantine Bureau, Jiangsu Province, P.R. China



Set up

■ Intro on CyHV-2

1. **Importation of CyHV-2** infected goldfish from Third countries into NL and characterization of strains (T. Ito, J. Kurita and O. Haenen, DAO resubmitted)



2. **CyHV-2 in wild gibel carp** (O. Haenen, M. Engelsma, T. Ito)



3. **A primary cell line** from allogynogenetic silver "crucian" carp, *Carassius auratus gibelio* brain (CrCB) (Duan Hongan)



CyHV-2: Goldfish herpes virus

- Double-stranded DNA virus, ~290kbp, 170-200 nm Ø
- Synonym: goldfish haematopoietic necrosis virus (GHNV)
- Susceptible species and where:
- **Goldfish *Carassius auratus***: Japan (Jung & Miyazaki 1995), USA (Groff et al. 1998, Goodwin et al. 2006), Taiwan (Chang et al. 1999), Australia (Stephens et al. 2004), New Zealand (Hine et al. 2006), UK (Jeffery et al. 2007), France (Boitard et al. 2016), and Switzerland (Giovannini et al. 2016), Netherlands (Haenen et al.), ...
- **Prussian/gibel carp: *Carassius (aur.) gibelio***: China (Wang et al. 2012; Duan Hongan, 2017), Czech Republic (Daněk et al. 2012), Netherlands (Haenen et al., 2016), ...
- **Crucian carp: *Carassius carassius*** populations in Italy (Fichi et al. 2013, 2016), ...



CyHV-2: Goldfish herpes virus (2)

- At water temp: 15-25°C
- Mortality: may reach 100%
- Diagnostic method: conv. PCR targeting the CyHV-2 helicase gene (Waltzek et al. 2009), a.o.
- Virus isolation: on expt. cell lines, like goldfish fin (**GFF**) and Standard Ryukin Takafumi (**SRTF**) cell lines (Ito et al. 2013); or "crucian" (Prussian) carp brain primary cell line **CrCB** (Duan Hongan et al., 2017), a.o.
- Transmission: horizontal
- Vaccine? Only experimental (T. Ito et al.),...



1. Importation of CyHV-2 from 3rd countries into NL (Schiphol project)

(Ito et al., 2017, DAO, accepted)



- Dutch Veterinary Service (NVWA)
- Nov 2014 - Feb 2015, sampled at 4 importers in NL
- 50 batches of freshwater ornamental fish from 12 third countries → 2 live fish/batch to our lab
- Necropsy, and sampling for bacteriology, AMR tests, and for virology



Sampling at our Dutch lab



From 8 batches goldfish or shubunkin, the kidney was sampled and stored at -80°C, for CyHV-2 virus isolation, PCR, and pathogenicity tests in Japan, and sequencing (lab NRIA, in kind cooperation with T. Ito et al.)

Goldfish, CyHV-2 infected

Light exophthalmus, ascites, haemorrhages, kidney inflammation



However, this study: Most goldfish/shubunkin showed no clinical signs



Experimental work at NRIA in Matsusaka City, Japan, with Dr. T. Ito and coworkers



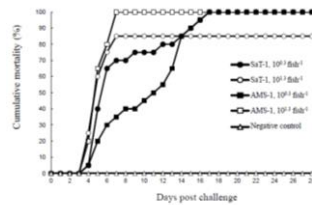
Methods and Results (Ito et al., 2017, DAO, accepted)

- 8 batches goldfish imported into the Netherlands by airfreight from Asia and the Middle East ⇒ tested for virus (conv. PCR Waltzek et al. 2009) & virus isolation
- **4 of 8 positive** by PCR (1x from a CyHV-2 disease case at the Dutch importers quarantine facility)
- **virus isolation:**
 - **In vitro: 0 of 4 positive**
 - **In vivo** in goldfish (i.p. kidney homogenate) ⇒ **1 of 4 positive** (sample **AMS-1**, mortality) ⇒ kidney of every goldfish positive in virus isolation *in vitro*, and for infection trial in goldfish (from GFF cells)
 - Ref. strain Japan **SaT-1** (Ito et al., 2013) used



Results infection trial with AMS-1 & SaT-1 (CyHV-2)

Group	Infectious dose (TCID ₅₀ fish ⁻¹)	Cumulative mortality (%)	Dead fish		Surviving fish	
			PCR	Virus re-isolation	PCR	Virus re-isolation
SaT-1	10 ^{0.1}	100	20/20	20/20	-	-
	10 ^{1.3}	85	17/17	17/17	3/3	0/3
	10 ^{0.3}	100	20/20	20/20	-	-
AMS-1	10 ^{0.3}	100	20/20	20/20	-	-
	10 ^{1.3}	100	20/20	20/20	-	-
Negative control		0	-	-	0/20	0/20



(Ito et al., 2017, DAO, accepted)

Results (2) and conclusions (Ito et al., 2017, DAO, accepted)

- Pathogenicity of the AMS-1 isolate to naïve goldfish was **similar** to that of the Japanese reference isolate (SaT-1)
- The viral titre of the AMS-1 isolate in GFF cells at several temperatures was **similar** with that of the SaT-1 isolate
- Sequence analysis of CyHV-2 strains (this study and previous reports): ≥ 6 different lengths in the mA region ⇒ **tentatively ≥ 4 genotypes** (data soon published)
- Various CyHV-2 strains spread through global trade of sometimes apparently clinically healthy goldfish
- I acknowledge Dr. Ito and colleagues for this study
- For those interested: Procedure of development of fish cell lines is available (T. Ito)



2. CyHV-2 in wild gibel carp (O. Haenen, M. Engelsma, T. Ito)

- **May 2015**, epizootic with high mortality in adult wild gibel carp in freshwater lake De Wollebrand, Westland, in the Netherlands, at 20°C

small mediahype...

1500 kg gibel carp died...



Realtime qPCR strong + (Goodwin et al. 2006)

⇒ **Archive** wild gibel carp samples from high mortalities at 20-25°C with haemorrhagic eyes, thick slime, loosening skin, and gill inflammation with hypertrophy tested :

- **May 2011**, 2500 gibel carp died: **qPCR strong +**

- **Aug 2011**, high mortality: **qPCR strong +**



Gibel carp, *Carassius auratus gibelio*

- is thought to be an ancestral species of goldfish originating from China, which was introduced into the Netherlands since the 17th century



28 Aug 2016 wild gibel carp, W-NL

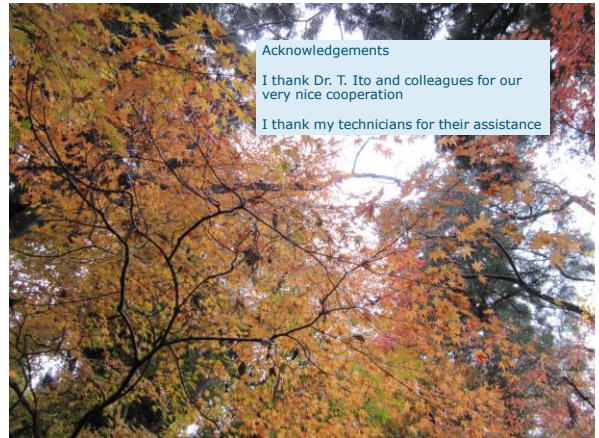
- Bleeding gills, sudden surfacing, death → high mortality
- Haemorrhages in head, gills pale and necrotic
- Liver pale and loose, pale and swollen kidney, gut slightly haemorrhagic, petechial haemorrhages in muscle and swimbladder
- Water temperature >16°C
- qPCR: Ct 12 = high amount



Conclusions

- CyHV-2 is present in global trade of often healthy looking goldfish, a risk
- CyHV-2 is devastating for wild gibel (Prussian) and crucian carp populations
- Diagnostic methods are partly available ((q)PCRs, for virus isolation you need special cells, no serology yet)
- No vaccine available yet, wild fish not vaccinable
- **Is CyHV-2 disease alarming for Europe/world? Action needed, projects?**

....DISCUSSION



Acknowledgements

I thank Dr. T. Ito and colleagues for our very nice cooperation

I thank my technicians for their assistance