

Emergence of a Cyprinid Herpesvirus (CyHV-2) in goldfish *Carassius auratus* in the UK

Background and introduction

Cyprinid herpesvirus II (CyHV-2) has been described under other names such as Goldfish herpesvirus (GHV) and Herpesviral Haematopoietic Necrosis virus (HHNV). The first recorded outbreak was in the Aichi prefecture, Japan in 1992 (7). Since then, further outbreaks have been reported in Taiwan 1999 (1), USA 1997, 2002, 2003 and 2004 and Australia 2003 (8).

The CyHV-2 virus was first suspected and later confirmed in the UK in 2002 in connected retail outlets. Further outbreaks occurred in 2003, 2004 and 2005 in some of these outlets and also in the holding and distribution centre. These outbreaks resulted in low-level mortalities, peaking at approximately 20%. However, further outbreaks were confirmed in unconnected hobbyists ponds in England and Wales in 2005 and 2006 with higher mortality rates.

Analysis of archive fixed-tissue has identified CyHV-2 in samples from a site in England as long ago as 1996.

Disease identification

Clinical disease

The clinical disease is usually observed after water temperatures have increased to above 15°C. The main symptoms include lethargy, anorexia and pale patches on the skin and gills. Symptoms are not unlike KHV disease in koi carp.



Figure 1: Goldfish showing extensive pale patches on the gills sampled during the 2004 investigation.



Figure 2: Goldfish showing enlarged spleen with white granular nodules sampled during the 2004 investigation.

Internally the symptoms include a pale liver, enlarged kidney and spleen, and white nodules within the spleen.

Pathology

Histological examination of tissues has revealed several typical pathologies.

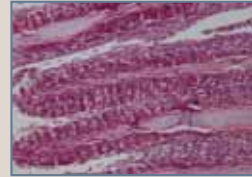


Figure 3: Generalised fusion of secondary gill lamellae with necrosis and sloughing of epithelial cells.

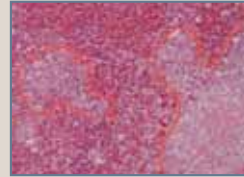


Figure 4: Low power section of spleen with two principle focal lesions stained with H&E. The smaller lesion (left) is characterised by infiltration of lymphoid cells, compared with the larger lesion (right) which is comprised almost exclusively of necrotic pale staining cells.

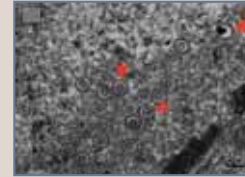


Figure 5: Higher power image of infected cells showing the developing virions and a fully formed particle within the nucleus. The fully formed virion (arrow) shows an electron dense core and outer membrane. The core of the particle is not centralised and appears dome shaped extending out from the membrane (bar = 100nm).

Cell culture isolation

The virus has been isolated in koi fin (KF) cells but has tended to lose infectivity when sub-cultured. There has been no growth on Epithelioma Papulosum Cyprini (EPC), Fat Head Minnow (FHM) or Goldfish fin (GFF) cells.

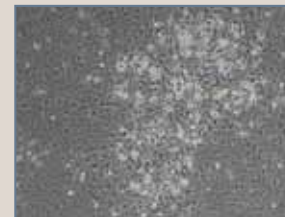


Figure 6: Cytopathic effect observed in koi fin cells inoculated with extracts of gill tissue.

PCR Assay and sequence identification

As cell culture isolation has proved problematic, identification of CyHV-2 is carried out by the use of Polymerase Chain Reaction (PCR)

PCR tests are run on pools of tissue from viscera (kidney, spleen and brain) and gills. Sequencing of the virus found in 2003 and 2004 showed a 100% nucleotide sequence identity with the published sequence for CyHV-2 (9).



Figure 8: ABI genome sequencer.



Figure 7: DNA bands amplified by PCR are excised from gels for further sequencing.

Recent studies using quantitative real-time PCR show that CyHV-2 is widespread in the USA and it is suggested that CyHV-2 is likely to be an important, but rarely detected, pathogen world-wide (5).

CyHV-2 transmission trials

Transmission trials carried out at Cefas have demonstrated that the virus is pathogenic for goldfish *Carassius auratus*. The following figure shows the results of a tank trial on the pathogenicity of CyHV-2, isolate UK-H278, at 20°C.

In the UK, the native crucian carp *Carassius carassius* is under increasing threat from direct competition with goldfish and carp through, hybridisation with these species, and habitat degradation (2). Consequently, there was concern about the potential impact of CyHV-2 on this species.

In Cefas transmission trials, CyHV-2 was not pathogenic for Crucian carp (10)

Subsequent PCR tests detected CyHV-2 in all goldfish mortalities but not in crucian carp.

These trials again demonstrated the difficulty of recovering this virus from infected fish by culture isolation.

Recent work in the USA on goldfish fry hatched in well water from eggs treated with iodophore, formalin, and/or potassium permanganate, detected CyHV-2. This suggests that vertical transmission occurs and that the virus may be within the eggs rather than on the surface (3).

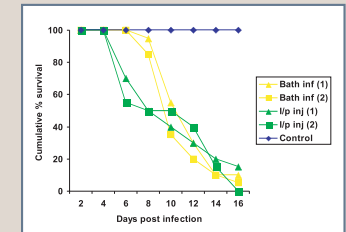


Figure 9: Susceptibility of goldfish to CyHV-2 isolate UK-H278.

Further studies

Whilst research has shown that the virus is not pathogenic for our threatened native crucian carp, concern has been expressed that hybridisation might provide an indirect path for viral entry to this species.

Cefas priorities for further work on CyHV-2 are: -

Further transmission trials on: -

- Goldfish
- Goldfish x Crucian carp hybrids
- Goldfish x Common carp hybrids
- Other cyprinid species

Development of PCR-based molecular methods to detect CyHV-2 in carrier fish (more sensitive screening)

Acknowledgement

Research on CyHV-2 and other emerging diseases is funded through Defra contract F1166

References

- (1) Chang P.H., Lee S.H., Chiang H.C. & Jong M.H. (1999) Epizootic of Herpes-like virus infection in goldfish, *Carassius auratus* in Taiwan. *Fish Pathology* **34**, 209-210.
- (2) Environment Agency, crucian carp field guide.
- (3) Goodwin A.E., Merry G. & Sadler, (2006) Detection, Epidemiology, and Vertical Transmission of Cyprinid Herpesvirus-2 Infections of Goldfish. Abstract, 5th International Symposium on Aquatic Animal Health, San Francisco, Sept. 2006.
- (4) Goodwin A.E., Khoo L., LaPatra S.E., Bonar A., Key D.W., Garner M., Lee M.V. & Hanson L. (2006) Goldfish haematopoietic necrosis herpesvirus (Cyprinid Herpesvirus 2) in the USA: Molecular confirmation of isolates from diseased fish. *J. Aquat. Anim. Health* **18**, 11-18.
- (5) Goodwin A.E. (2005) Herpesviral haematopoietic necrosis of goldfish: Detection of Cyprinid herpesvirus 2 (CyHV-2) by quantitative PCR and clinical evidence that CyHV-2 is an important and widespread pathogen. Abstract (O-588), EAAP 120th International Conference, Copenhagen, Sept. 2005.
- (6) Groff J.M., LaPatra S.E., Munn R.J. & Zinkl J.G. (1998) A viral epizootic in cultured populations of juvenile goldfish due to a putative herpesvirus etiology. *J. Vet. Diagnostic Invest* **10**, 375-376.
- (7) Jung S.J. & Miyazaki T. (1995) Herpesviral haematopoietic necrosis of goldfish, *Carassius auratus*, (L.). *J. Fish Dis.* **18**, 211-220.
- (8) Stephens F.J., Raidal S.R. & Jones B. (2004) Haematopoietic necrosis in a goldfish (*Carassius auratus*) associated with an agent morphologically similar to herpesvirus. *Australian Vet J.* **82**, 167-169.
- (9) Waltzek T.B., Kelley G.O., Stone D.M., Way K., Hanson L., Fukuda H., Hirono I., Aoki T., Davison A.J. & Hedrick R.P. (2005) Koi herpesvirus represents a third cyprinid herpesvirus (CyHV-3) in the family Herpesviridae. *J. Gen. Virol.* **86**, 1659-1667.
- (10) Way, K., Personal Communication.