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

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).

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:
  • • •
• Identification of CyHV-2 is carried out by the use of Polymerase Chain Reaction (PCR)
• Sequencing of the virus found in 2003 and 2004 showed a 100% nucleotide sequence identity with the published sequence for CyHV-2 (9).

CyHV-2 transmission trials

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 Euphithelia 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.

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).

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) and Sequencing of PCR products.

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.

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

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.

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

References

(2) Environment Agency, crucian carp field guide.

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.

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