

2.2.11 Goldfish Herpesviral Hematopoietic Necrosis Disease

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A. Name of Disease and Etiological Agent

Goldfish Herpesviral Hematopoietic Necrosis Disease (HVHN) is caused by a herpes-like virus classified as *Cyprinid Herpesvirus 2* (CyHV-2) by the International Committee on the Taxonomy of Viruses. The DNA sequence of CyHV-2 is most similar to those of CyHV-1 (the carp pox virus) and CyHV-3 (the koi herpesvirus).

B. Known Geographical Range and Host Species of the Disease

1. Geographical Range

There have been published accounts of HVHN in goldfish from Japan, the US, Taiwan, and Australia. There are unpublished reports of CyHV-2 in Europe. Given the known distribution of the virus and the level of international commerce in goldfish, it seems likely that the virus is present in most goldfish producing countries.

2. Host Species

The CyHV-2 virus and HVHN disease have only been reported in goldfish (*Carassius auratus*), but diagnostic tools sufficient to detect carrier states in other species have only recently been developed. In experimental infections, goldfish X koi (*Cyprinus carpio*) hybrids carry the virus but do not develop the disease. Koi did not carry the virus or develop disease.

C. Epizootiology

Despite the wide geographic distribution of CyHV-2 and the global commerce in goldfish, reports of HVHN are quite rare. This may have been partially due to technical challenges that made definitive diagnosis difficult prior to 2006. The virus does produce very high mortality during epizootics and some small breeders and private pond owners have experienced devastating losses on an annual basis. The virus can be detected in healthy-looking fish tested by PCR as part of fish health surveys. The best evidence is that the virus is widespread, but only causes disease under certain conditions. Those exact conditions are unknown, but epizootics are most common during the spring and fall when water temperatures are above 18 °C. Significant outbreaks have occurred at temperatures as high as 28°C and as low as 15°C.

D. Disease Signs

1. Behavioral Signs

Fish may become lethargic and rest on the bottom. Increased ventilation rates and loss of equilibrium have also been described.

2. Gross Signs

Gills may be generally pale or there may be pale patches on the skin and gills similar to those reported in koi infected by KHV (CyHV-3).

3. Microscopic Signs

Histology of head kidneys from goldfish with acute HVHN reveals extensive necrosis of hematopoietic cells (figure 1). Nuclei of hematopoietic cells are sometime enlarged with marginated chromatin. Similar lesions may be seen in the spleen (figure 2). Many fish also have inflammation and necrosis in the sub-mucosa of the intestine (figure 3). Other lesions have been reported but are not consistently noted.

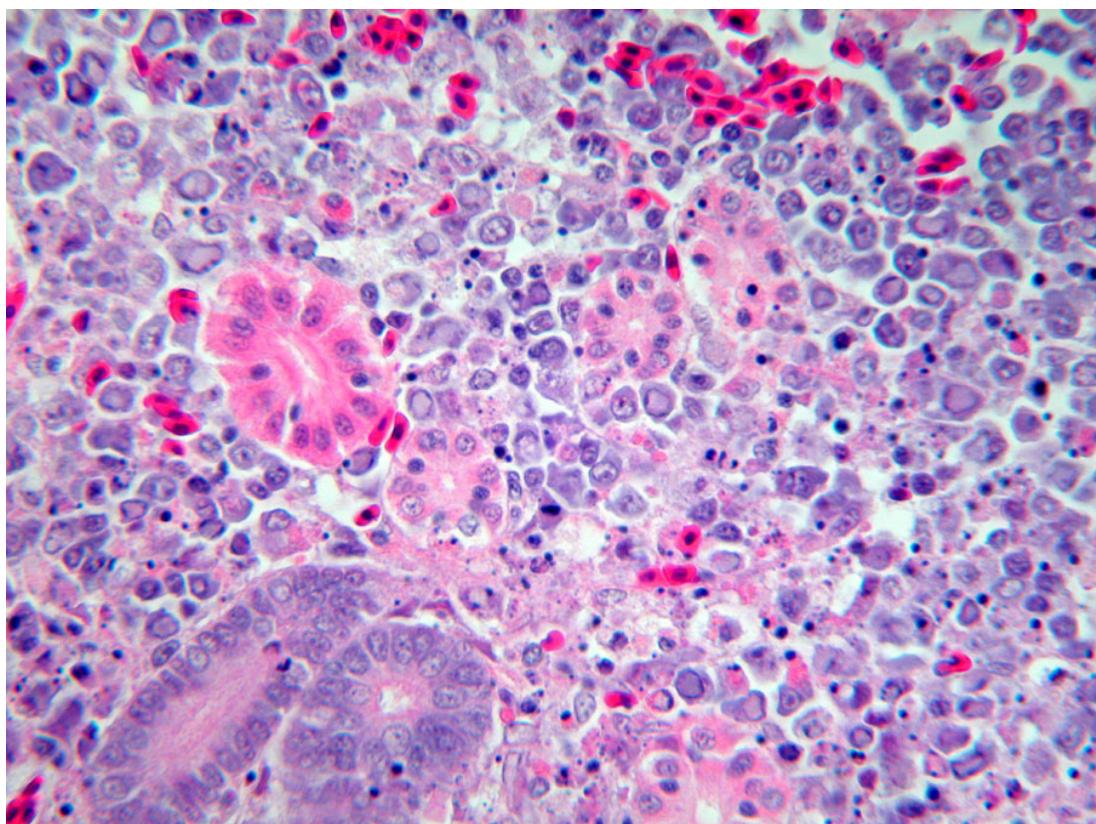


Figure 1: Necrosis of hematopoietic cells in the kidney of a goldfish with Goldfish Herpesviral Hematopoietic Necrosis Disease. Many pyknotic nuclei are evident. Other nuclei are enlarged with marginated chromatin. Picture by Dr. Lester Khoo.

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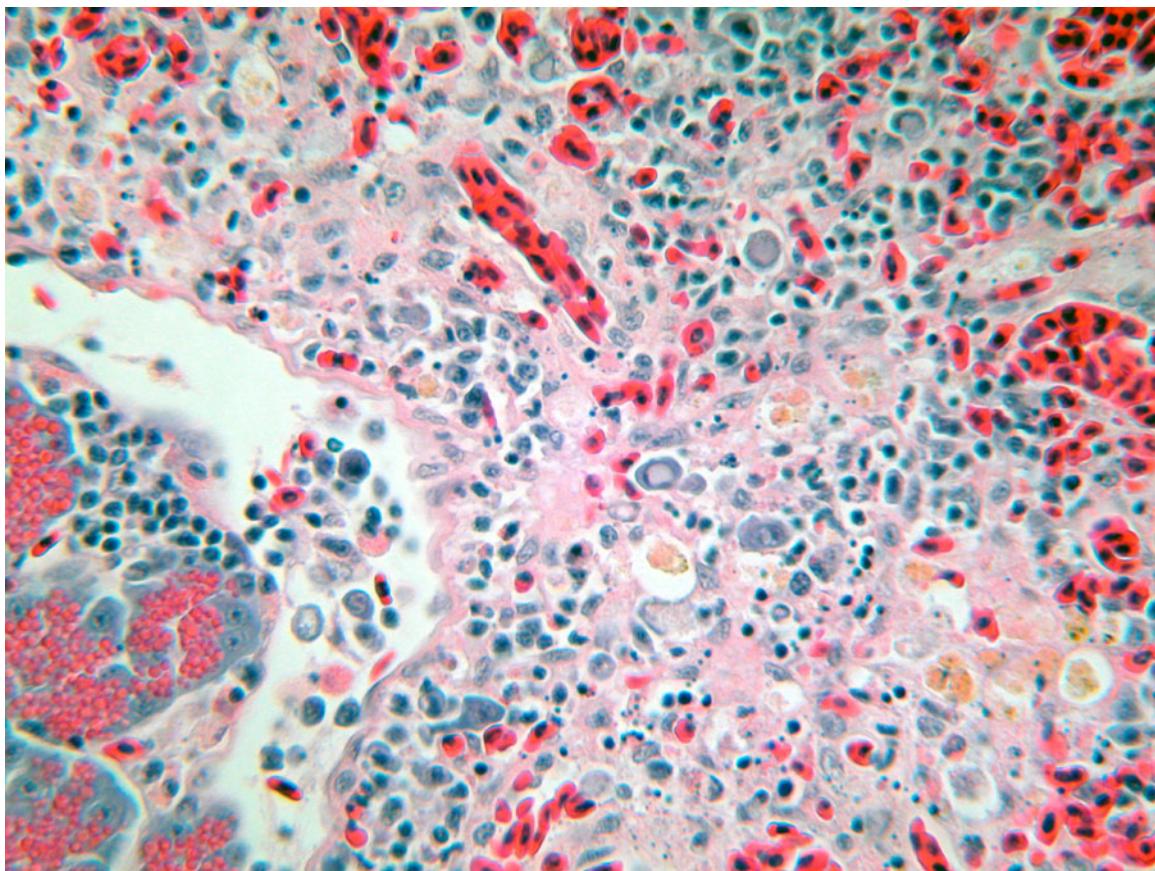


Figure 2: Necrosis of hematopoietic cells in the spleen of a goldfish with Goldfish Herpesviral Hematopoietic Necrosis Disease. Many pyknotic nuclei are evident. Other nuclei are enlarged with marginated chromatin. Picture by Dr. Lester Khoo.

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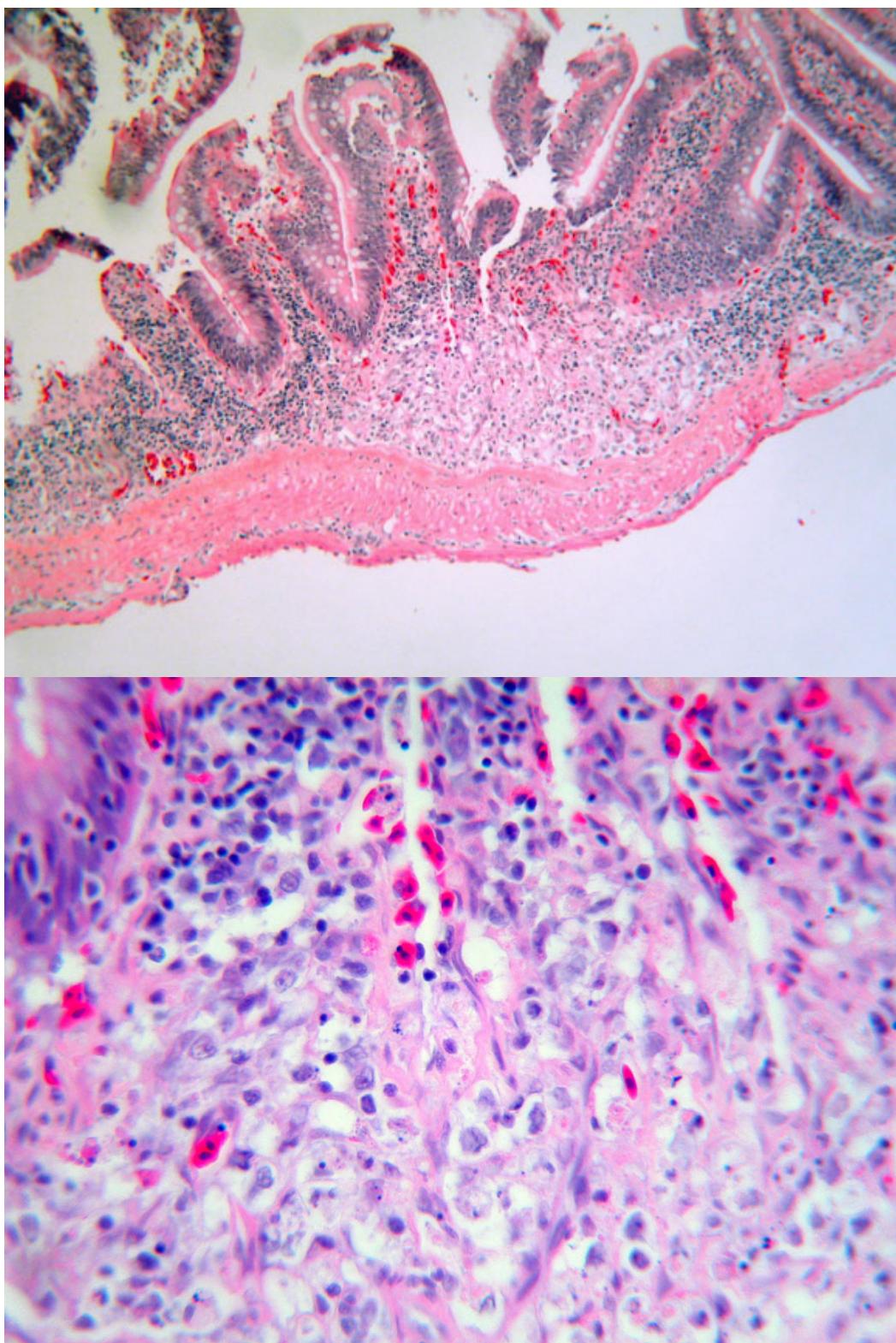


Figure 3: Necrosis of the submucosa in the intestine of a goldfish with Goldfish Herpesviral Hematopoietic Necrosis Disease. The bottom frame is a higher magnification of the focal area of necrosis evident near the center of the top frame. Picture by Dr. Lester Khoo.

E. Disease Diagnostic Procedures

The CyHV-2 virus is difficult to propagate in cell culture so successful diagnosis relies on histology or molecular methods. Most laboratories see only transient or no cytopathic effects in cell cultures. If culture is attempted, rare success is reported with the koi fin cell line (KF-1). There is a report of successful culture in a goldfish fin cell line in Japan, but that cell line is not readily available and laboratories attempting detection of CyHV-2 in that cell line often report failures. There are no antibodies available for serological testing.

1. Presumptive Diagnosis

a. Histology

Severe necrosis of the hematopoietic tissues in the head kidney and spleen is the most dependable histological lesion (figures 1 and 2). Necrosis and inflammation of the intestinal sub-mucosa is also common (figure 3).

b. Polymerase Chain Reaction

PCR may be used to detect CyHV-2 directly in the tissues of fish (Goodwin et al. 2006). However, the assay is very sensitive and will easily detect CyHV-2 in carrier fish. Care must be taken to insure that PCR-positive fish have acute HVHN and are not CyHV-2 carriers dying from another cause. Standard PCR is carried out as described below, except that the fluorescent probe is omitted and the 92 bp amplicon is resolved using a 1.5% agarose gel.

2. Confirmatory Diagnosis

a. Confirming a Histological Diagnosis

When histological lesions consistent with HVHN are evident, the presence of CyHV-2 should be confirmed by PCR or by real time quantitative PCR.

b. Confirming a diagnosis made by standard PCR

The only virus known to cross react with the CyHV-2 primer set is CyHV-1, but this pathogen has never been reported in goldfish. The primary risk involved in diagnosis by standard PCR alone is that the detection of CyHV-2 will lead to a diagnosis of HVHN when the fish was actually a CyHV-2 carrier fish moribund due to infection by another pathogen. Thus, other infectious or environmental causes should be ruled and the diagnosis confirmed either by histology or by quantitative real-time PCR. In the event that the clinical signs or PCR products are in any way atypical, further confirmation of CyHV-2 can be obtained by sequencing the PCR product and comparing it to the sequences deposited in GenBank (accession number AY939863).

1. DNA Extraction from Fish Tissues

DNA is prepared from a pool of kidney and spleen tissues using either conventional chloroform isoamyl alcohol extraction or by commercially available kits (Dneasy Tissue Kit, Qiagen Inc., CA, USA). The DNA concentration of the purified DNA is determined by spectrophotometry.

2. PCR Conditions

The conditions that provide quantitative amplification of the specific 170 bp fragment are as follows:

Cocktail: For 25 µl reactions combine 5 µl of template (concentration 50-800 µg/µl), 1.0 µl each of the forward and reverse primers (10µM each), 1.0 µl of FAM-labeled fluorescent probe (10 µM), 13 µl of 2X supermix (Bio-Rad Laboratories, Hercules, CA),

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and 4.0 µl of water. If compatible with the PCR machine, 12.5 µl reactions may also be successfully used.

Forward primer: 5'- TCGGTTGGACTCGGTTGTG – 3'

Reverse primer: 5'- CTCGGTCTTGATGCGTTCTG – 3'

Fluorescent Probe: 5'- FAM-CCGCTTCCAGTCTGGGCCACTACC- BHQ1– 3'

Standards and Controls: Given that CyHV-2 has not been successfully propagated in vitro, the only suitable positive controls are DNA derived from the tissues of fish with natural CyHV-2 infections. Standards are most easily produced by cloning the product of a positive CyHV-2 reaction and then using a known concentration of purified plasmid to establish a standard curve.

Cycling conditions: Quantitative reactions with the fluorescent probe must be run on a PCR machine with the capability to perform and analyze real time PCR reactions utilizing fluorescent reporters. The cycle consists of an initial incubation of 2 min at 95°C followed by 35 cycles of 45 s at 58°C, 45 s at 72°C, and 30 s at 95°C, and a final extension of 2 min at 72°C

3. **Interpreting the Results:** The copy number per reaction in fish samples is determined from the standard curve and normalized as the number of copies per µg of template DNA used. Most brood fish and carrier fish have from 0-50,000 copies of the CyHV-2 genome per µg template DNA. Fish with clinical HVHN may have millions of copies per µg template DNA. These ranges provide rough guidelines for differentiating between carriers and acutely diseased fish, however, it must be recognized that even in active infections the copy number varies as the disease progresses and there is the possibility that copy number may increase in fish in an immunocompromised state resulting from infection by another pathogen or from environmental conditions.

F. Procedures for Detecting Subclinical Infections

Subclinical infections are detected in healthy fish by quantitative real time PCR done directly on fish tissues. The calculated sensitivity of the PCR assay approaches 1 copy of the viral genome.

G. Procedures for Detecting Prior Exposure

There are no published serological methods for detecting antibodies against CyHV-2 in the blood of fish surviving HVHN. Quantitative PCR does detect the virus in healthy fish, including mature broodfish from sources where the virus is prevalent and thus may be useful in detecting populations of fish exposed to CyHV-2.

H. Procedures for Transportation and Storage of Samples

Samples for histology should be collected and fixed according to standard methods. Tissues for PCR may be stored in 95% ethanol or frozen for later DNA isolation.

References

- Chang, P. -H., S. -H. Lee, H. -C. Chiang, and M. -H. Jong. 1999. Epizootic of herpes-like virus infection in goldfish, *Carassius auratus* in Taiwan Fish-Pathology 34(4): 209-210.
- Goodwin, A. E., L. Khoo, S. E. LaPatra, C. Bonar, D. W. Key, M. Garner, and L. Hanson. 2006a. Goldfish Hematopoietic Necrosis Herpesvirus (*Cyprinid herpesvirus 2*) In the USA: Molecular Confirmation of Isolates from Diseased Fish. Journal of Aquatic Animal Health 18:11-18.
- Goodwin, A. E., G. E. Merry, and J. Sadler. 2006b. Detection of the herpesviral hematopoietic necrosis disease agent (*Cyprinid herpesvirus 2*) in moribund and healthy goldfish: Validation of a quantitative PCR diagnostic method. Diseases of Aquatic Organisms 69:137-143.
- Groff, J. M., S. E. LaPatra, R. J. Munn, and J. G. Zinkl. 1998. A viral epizootic in cultured populations of juvenile goldfish due to a putative herpesvirus etiology. Journal of Veterinary Diagnostic Investigations 10: 375-378.
- Hedrick, R. P., T. B. Waltzek, and T. S. McDowell. 2006. Susceptibility of Koi Carp, Common Carp, Goldfish, and Goldfish × Common Carp Hybrids to Cyprinid Herpesvirus-2 and Herpesvirus-3. Journal of Aquatic Animal Health 18:26-34.
- Jung, S. J., and T. Miyazaki. 1995. Herpesviral haematopoietic necrosis of goldfish, *Carassius auratus* (L.) Journal of Fish Diseases 18:211-220.
- Stephens, F. J., S. R. Raidal, and B. Jones. 2004. Haematopoietic necrosis in a goldfish (*Carassius auratus*) associated with an agent morphologically similar to herpesvirus. Australian Veterinary Journal 82:167-169.
- Waltzek, T. B., G. O. Kelley, D. M. Stone, K. Way, L. Hanson, H. Fukuda, A. J. Davison, and R. P. Hedrick. 2005. Koi herpesvirus represents a third cyprinid herpesvirus (CyHV-3) in the family *Herpesviridae*. Journal of General Virology 86: 1659-1667.