

2.2.10 Koi Herpesvirus Disease

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A. Name of Disease and Etiological Agent

Koi herpesvirus disease (KHVD) is caused by a recently recognized herpes-like virus (KHV) also known in Israel as carp nephritis and gill necrosis virus (CNGV). While clearly different from herpesviruses found in mammals, birds and reptiles, KHV is related to a group of herpes-like viruses found in fish and amphibians currently classified in the family Herpesviridae.

B. Known Geographical Range and Host Species of the Disease

1. Geographical Range

The virus has been detected in koi or common carp from USA, Israel, England, Denmark, Germany, Netherlands, Luxembourg, Belgium, France, Austria, Switzerland, Italy, Poland, China, Taiwan, Japan, Indonesia and South Africa.

2. Host Species

To date only *Cyprinus carpio* (koi and common carp) is known to be susceptible to KHV infection. Other carps and goldfish are resistant to infection and those tested appear not to be capable of carrying the virus as a subclinical infection (Perelberg et al. 2003). The virus has been detected principally among ornamental fish and koi in particular but more recently KHV has been detected in wild common carp in Europe and South Carolina, U.S.A.

C. Epizootiology

The origins of KHV are somewhat obscure but the cases in Germany in 1996–1997 maybe the first recorded observations (Bretzinger et al. 1999). Isolation of the virus and confirmation that it was the cause of the disease were completed in 1998 (Hedrick et al. 1999, 2000). Young koi and common carp are most susceptible although outbreaks and high mortality (90 %) have been observed in larger and adult fish. Mortality begins approximately 7–14 days following contact with the virus most often via a recently introduced fish to the pond (koi) with one of two common outcomes: 1) the introduced fish dies shortly after introduction and spreads the virus to the naïve population or 2) the introduced fish stays healthy yet the naïve fish succumb. The movement of the virus with suspected carriers has been the principal means by which the virus has spread so rapidly at both the regional and global scales.

D. Disease Signs

1. Behavioral Signs

Infected fish may demonstrate a hypersecretion of mucus which may cloud the pond water early in infection. Later, fish are lethargic, may lose equilibrium and position in the water column and may demonstrate signs of suffocation (Walster 1999).

2. Gross Signs

A white patchy appearance to the epidermis and the gills may be the first observed external signs. Swelling and necrosis of gill filaments (Figure 1) are common and secondary infections with *Flavobacterium* spp. may occur. Diffuse hemorrhages on the epidermis may or may not be present. Eyes may demonstrate a sunken appearance. Internal signs include swelling of the kidney and spleen. There may or may not be hemorrhages evident in the body wall of the peritoneum.

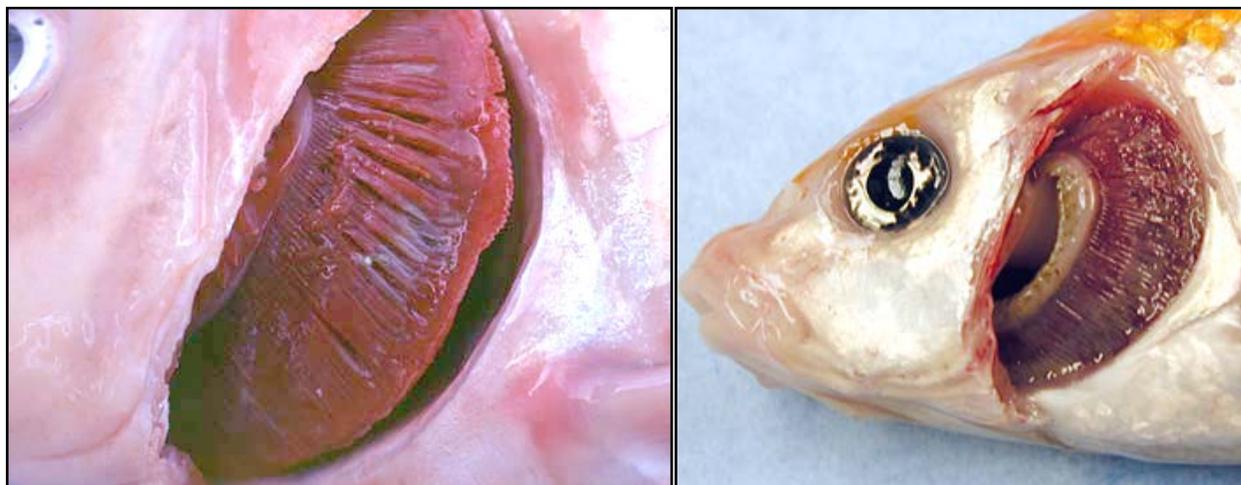


Figure 1. Gills of koi with KHVD showing (left) the swollen and fused lamellae and (right) white patchy appearance and necrosis progressing from distal to more basal portions of the lamella.

3. Microscopic Signs

There is both a hyperplasia and hypertrophy of gill epithelial cells. Inflammation and necrosis may also be evident in gill, kidney interstitium, parenchyma of the spleen and liver and the lamina propria of the intestine (Hedrick et al. 2000). Intranuclear inclusions are characterized by margination of the chromatin and a thickened nuclear membrane which surrounds a flocculent amphophilic to basophilic matrix (Figure 2). Intranuclear inclusions may not always be prominent in KHV infections.

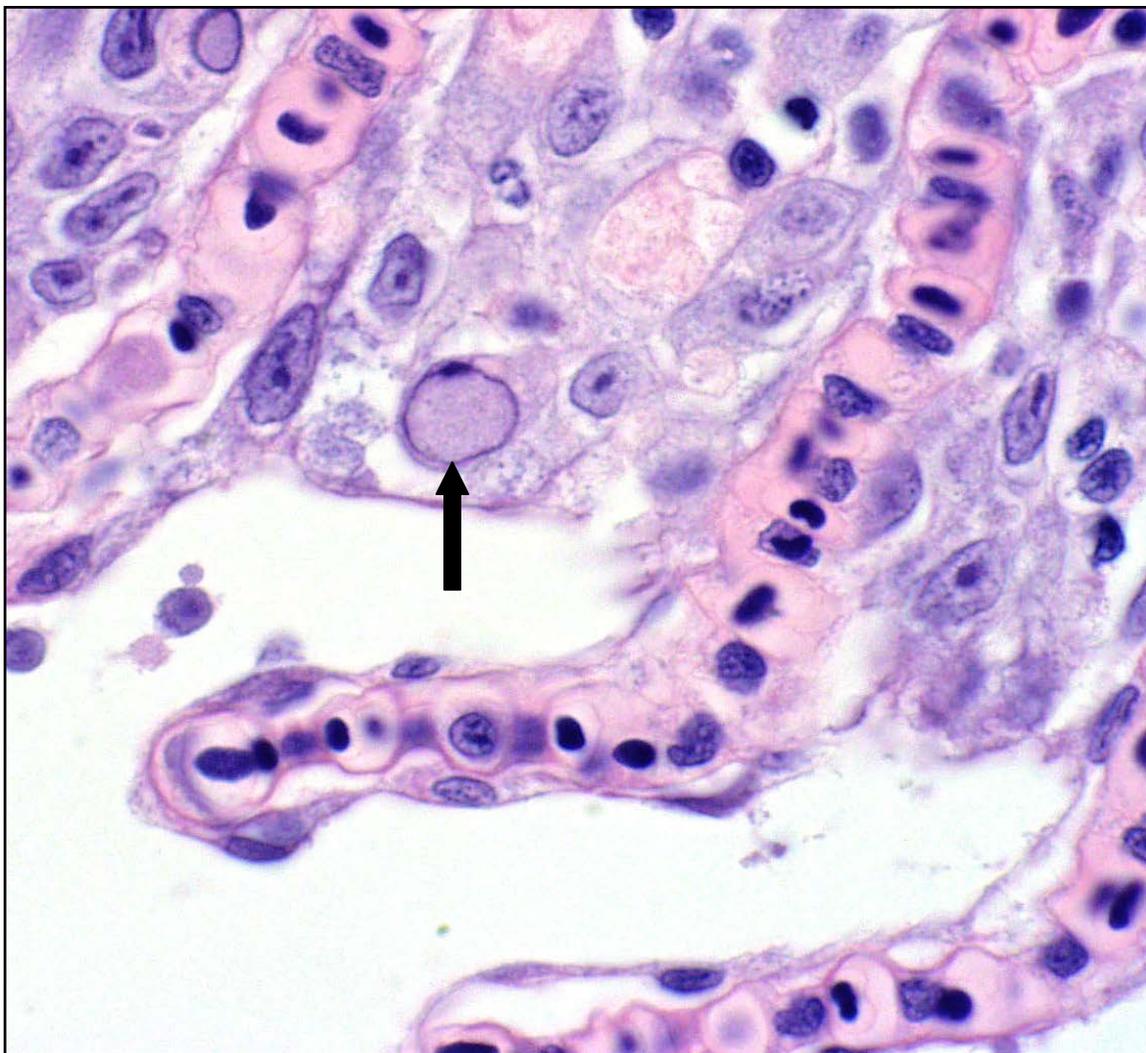


Figure 2. Secondary gill lamellae of a koi with KHVD. Adjacent lamellae are fused in the basal regions. Hypertrophy and necrosis of cells in the interlamellar region is evident as is the enlarged nucleus with a thickened membrane surrounding an amphophilic to basophilic matrix (arrow).

E. Disease Diagnostic Procedures

The diagnosis of KHVD is dependent upon demonstration of the clinical signs typical for the disease among koi or common carp at water temperatures between 16–28°C and presence of evidence for KHV either directly by isolation of the virus in cell culture or viral DNA by PCR. Confirmation that an isolated virus is KHV is accomplished by PCR. Immunostaining or indirect fluorescent antibody confirmation of infected cell cultures with anti-KHV rabbit antibodies are known but the antibodies are not routinely available nor has the test procedure been published.

Sampling Criteria

Most outbreaks of KHVD in the northern hemisphere occur during the spring and autumn when water temperatures are between 23–26°C. Infections can occur at any time of the year when water temperatures are between 16–28°C. The ability to routinely detect the virus at temperatures above or below these limits is difficult or impossible with current methods. Samples taken from the gills, kidney and spleen are deemed the most valuable for isolation of the virus or detection of viral DNA.

1. Presumptive Diagnosis

a. Clinical Signs

Lethargy and signs of suffocation and white patches on the gill and skin, gill necrosis, sunken eyes, enlarged kidney and spleen.

b. Virus Isolation

Two available cells lines are routinely used for virus isolation. The KF-1 cell line from koi fin (Hedrick et al. 2000) and the CCB line from common carp brain (Neukirch et al. 2000) support growth of KHV. The cells should be incubated at 20°C (Gilad et al. 2003). Cytopathic effects are characterized by the presence of small focally forming syncytia (fusing of the cytoplasm of several adjacent cells), enlarged nuclei with distinct nuclear membranes and the presence of numerous cytoplasmic vacuoles (Figure 3).

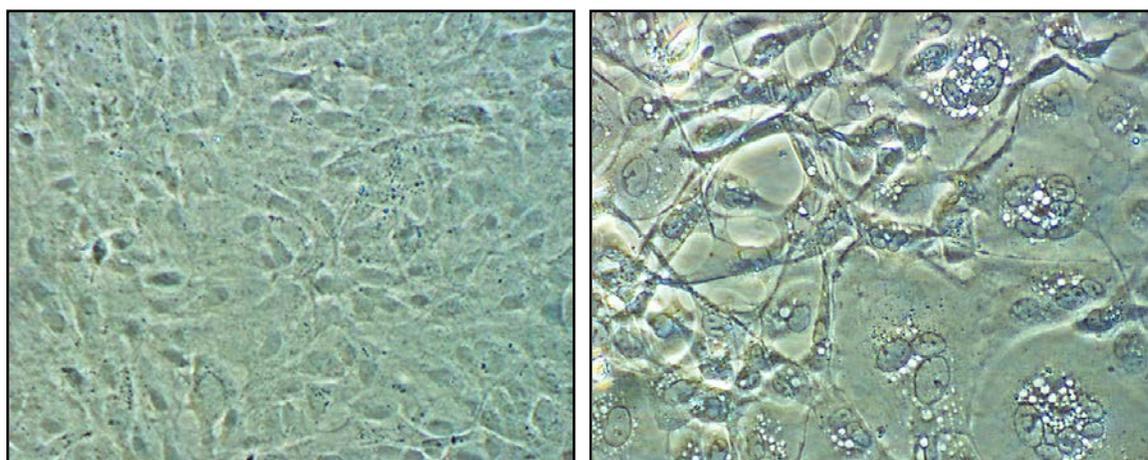


Figure 3. Cytopathic effects induced in KF-1 cells at 7 days post infection. Left panel is uninfected KF-1 cells and right panel is KHV-infected KF-1 cells. Note the presence of several enlarged nuclei within a large continuous cytoplasm (syncytium). Also evident is the intense vacuolation characteristic of KHV infected cells.

2. Confirmatory Diagnosis

a. Polymerase Chain Reaction

Confirmation that the virus present in KF-1 or CCB cells is KHV is based upon amplification of viral DNA using the polymerase chain reaction (PCR) assay. Two PCR tests are available, one described by Gilad et al. (2002) and the other by Gray et al. (2002). Primer sets and conditions for amplification can be found in these reports.

1. DNA Extraction from Fish Tissues

A pool of gill, kidney, and spleen tissues (ca. 1 g) are suggested samples for PCR analysis from suspect fish. Other suspect tissues such as gut or brain of infected fish may also be used. Tissues from moribund or freshly dead fish are preferred. The PCR can also be used on cells and supernatant from a suspect culture showing cytopathic. DNA for PCR is obtained from fish tissues or a 0.5 ml cell suspension (cells and media from a suspect culture) using a conventional extraction protocol (chloroform isoamyl alcohol) and then eluted in TE buffer (10 mM Tris-HCl, 1 mM EDTA pH 8) or sterile water. Alternatively, commercially available kits (Dneasy Tissue Kit, Qiagen Inc., CA, USA) can be used to extract DNA from tissues or cell suspensions following the manufacturer's

protocol. The DNA concentration is then determined by spectrophotometry. A total of 70-100 ng of DNA is used in each PCR using cycling conditions and primers as described below. DNA samples for PCR can be stored temporarily at 4°C or indefinitely at -70°C until used.

2. PCR Conditions

The conditions that provide amplification of the specific 484 bp fragment are as follows:

Cocktail: A 50 µl reaction mixture contains 2 mM MgCl₂, 1X PCR Buffer, 400 µM deoxynucleotide triphosphate, 30 pmol of each primer, and 1U *Taq* polymerase

Cycling conditions: 95°C for 5 min and then 39 cycles of 94°C for 1 min, 68°C for 1 min, 72°C for 30 s and followed by an extension at 72°C for 7 min.

Forward primer: KHV9/5F 5'-GAC GAC GCC GGA GAC CTT GTG-3';

Reverse primer: KHV9/5R 5'-CAC AAG TTC AGT CTG TTC CTC AAC-3'.

3. PCR Product Visualization and Sequencing

The 484 bp amplicon (derived from KHV DNA) can be visualized and compared to a commercially made DNA ladder following electrophoresis using a 1.5% agarose gel. Confirmation that the product is KHV can be obtained by DNA sequencing and comparison to reference sequences available in GenBank (AF411803).

F. Procedures for Detecting Subclinical Infections

There are no currently established criteria for detection of subclinical infections. The presence of viral DNA has been detected by PCR in fish recently recovered from clinical episodes of KHVD.

G. Procedures for Detecting Prior Exposure

An ELISA described by Ronen et al. (2003) has been utilized to demonstrate that fish exposed to the virus develop serum antibodies to KHV. This assay has not been widely field tested but is presumed to be a good indicator that fish have been exposed to the virus.

H. Procedures for Transportation and Storage of Samples

Samples collected from apparently moribund or dead fish should be packed on ice for shipment for virus isolation. Tissues for PCR analyses can be stored in 95% ethanol or kept frozen at ultracold temperatures. Virus isolation is difficult on fish dead for any period of time or following freezing.

References

- Bretzinger, A., T. Fischer-Scherl, M. Oumouma, R. Hoffmann, and U. Truyen. 1999. Mass mortalities in koi, *Cyprinus carpio*, associated with gill and skin disease. *Bulletin of the European Association of Fish Pathologists* 19:182-185.
- Gilad, O., S. Yun, K. B. Andree, M. A. Adkison, A. Zlotkin, H. Bercovier, A. Eldar, and R. P. Hedrick. 2002. Initial characteristics of koi herpesvirus and development of a polymerase chain reaction assay to detect the virus in koi, *Cyprinus carpio koi*. *Diseases of Aquatic Organisms* 48: 101-108.

- Gilad, O., S. Yun, K. B. Andree, M. A. Adkison, K. Way, N. H. Willits, H. Bercovier, R. P. Hedrick. 2003. Molecular comparison of isolates of an emerging fish pathogen, the koi herpesvirus, and the effect of water temperature on mortality of experimentally infected koi. *Journal of General Virology* 84: 1-8.
- Gray, W. L., L. Mullis, S. E. LaPatra, J. M. Groff, and A. Goodwin. 2002. Detection of koi herpesvirus DNA in tissues of infected fish. *Journal of Fish Diseases* 25: 171-178.
- Hedrick, R. P., G. D. Marty, R. W. Nordhausen, M. Kebus, H. Bercovier, and A. Eldar. 1999. An herpesvirus associated with mass mortality of juvenile and adult koi *Cyprinus carpio*. *Fish Health Newsletter, Fish Health Section, American Fisheries Society* 27:7.
- Hedrick, R. P., O. Gilad, S. Yun, J. V. Spangenberg, G. D. Marty, R. W. Nordhausen, M. J. Kebus, H. Bercovier, and A. Eldar. 2000. A Herpesvirus associate with mass mortality of juvenile and adult koi, a strain of a common carp. *Journal of Aquatic Animal Health* 12: 44-57.
- Neukirch, M., and U. Kunz. 2001. Isolation and preliminary characterization of several viruses from koi (*Cyprinus carpio*) suffering gill necrosis and mortality. *Bulletin of the European Association of Fish Pathologists* 21: 125-135.
- Perelberg, A., M. Smirnov, M. Hutoran, A. Diamant, Y. Bejerano, and M. Kotler. 2003. Epidemiological description of a new viral disease afflicting cultured *Cyprinus carpio* in Israel. *Israeli Journal of Aquaculture Bamidgeh* 55: 5-12.
- Ronen, A., A. Perelberg, J. Abramowitz, M. Hutoran, S. Tinman, I. Bejerano, M. Steinitz, and M. Kotler. 2003. Efficient vaccine against the virus causing a lethal disease in cultured *Cyprinus carpio*. *Vaccine* 21: 4677-4684.
- Walster, C. 1999. Clinical observations of severe mortalities in koi carp, *Cyprinus carpio*, with gill disease. *Fish Veterinary Journal* 3: 54-58.