KHV Testing

Dr. Ling Jin, Ph.D.
Carlson College of Veterinary Medicine
Oregon State University, Corvallis, Oregon

This mini-review on KHV latent infection is written for the koi hobbyist and koi breeders to better understanding of latent KHV infection diagnosis. Different diagnostic methods are described for screening KHV latent and active infections.

Introduction

Cyprinid herpesvirus 3 (CyHV-3), commonly known as koi herpesvirus (KHV), is a highly contagious pathogen of *Cyprinus carpio,* including common carp and koi (Gilad et al., 2002; Grimmett et al., 2006; Hedrick et al., 1990; Sunarto et al., 2011). KHV belongs to *Cyprinivirus* genus from the *Alloherpesviridae* family in the order of *Herpesvirales* (Aoki et al., 2007; Waltzek et al., 2005). KHV infection is characterized by white mottling of gills, gill hemorrhage, sunken eyes, and pale patches along with blisters on skin in koi (Gilad et al., 2002; Miyazaki et al., 2008; Pikarsky et al., 2004). Fry are more susceptible to KHV and may have 70-100% mortality upon exposure (Bondad-Reantaso et al., 2007; Gilad et al., 2002).

Similar to other members of *Herpesviridae* within the order of *Herpesvirales*, KHV can also establish life-long latency in symptomatic or asymptomatic recovered koi (Eide et al., 2011a; Eide et al., 2011b; Xu et al., 2013). During latency, the KHV genome is the only component of the virion (Fig. 1) hidden in the latently infected cells, i.e., there is no capsid or envelope present in the cells. KHV latency can often be reactivated under stress conditions, such as heat stress, poor water quality, shipping or injury. Infectious virions can be produced from KHV reactivation, which can cause disease and transmit to naïve fish (Eide et al., 2011c; St-Hilaire et al., 2005). Outbreaks of KHV often occur in the spring and summer months when water temperatures rise suddenly or during transportation (Marcos-Lopez et al., 2010; Omori and Adams, 2011). Latent infections can sometimes also be experimentally reactivated by raising tank water temperature by 1°C per day from 14°C to 23°C (Eide et al., 2011c). In general, koi with KHV latency can live healthy lives and may experience KHV reactivation without showing clinical signs. However, KHV carrier (latently infected) koi could die from KHV reactivation under certain stress conditions.

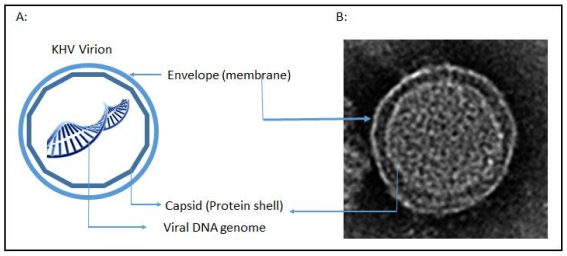

Figure 1. Structure of KHV virion. A: Schematic of KHV virion, which consists of viral genome encapsided by capsid and viral envelope (similar to celluar membrane). B: Herpesvirus vrion taken by Transmission Electron Microscope, which shows the capsid and envelope. The viral genome is inside of the capsid.

Active and latent KHV infection:

Active KHV infections occur when KHV virions are produced and cause damage to tissues (Fig. 2). Acute infections normally last 1-2 weeks. Following the acute infection, koi either survive or die. The survival rate of adult koi is higher than that of fry, which may have over 70% mortality. Some infected adult koi may show no signs of KHV infection. However, all exposed koi will become latently infected by KHV, no matter whether clinical signs are observed or not. The latently infected koi can live the same normal life as an un-infected koi and produce no KHV virions (Fig. 1). Koi with KHV latency are difficult to differentiate from KHV-free koi. During KHV latency, only the viral genome is maintained in the latently infected lymphocytes, which can be sampled from blood (Fig. 2). Upon exposure to KHV, koi also produce antibodies specific to KHV. The antibodies are normally detectable within 2-3 weeks to 12 months following initial infection (Adkison, et al., 2005)

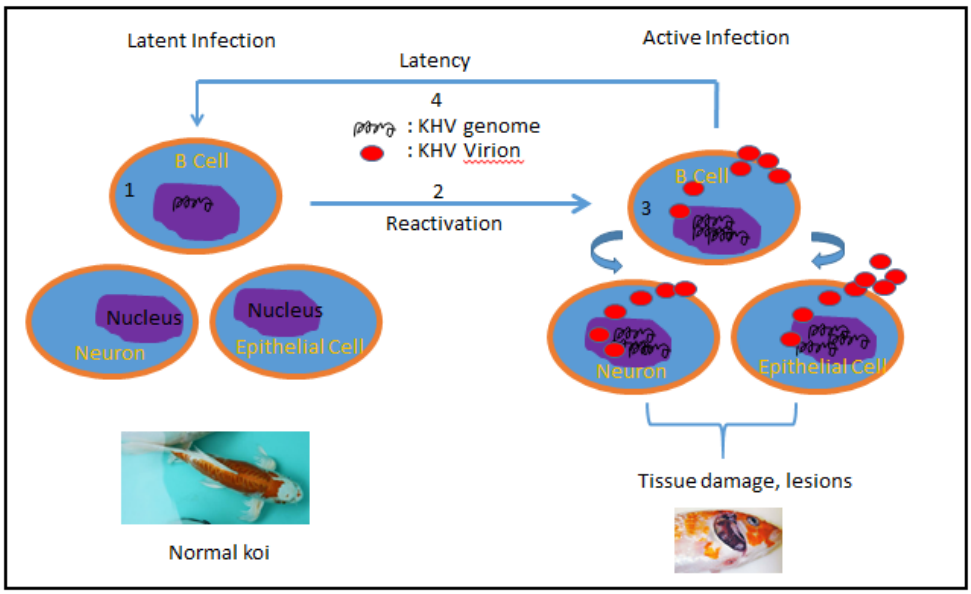

Figure 2. Latency and Reactivation cycle of KHV infection in a latently infected koi. 1. KHV genome resides in the B cell nucleus during latent infection. 2: Stressors, such as heat, injury and poor water quality, trigger KHV reactivation. 3: KHV virions (including viral genomes) are made during KHV reactivation and spread to permissive tissues, such as gills and brain, and cause tissue damages. 4. KHV infection is controled by the host immune system; KHV resumes latency and hides in the B cells again.

Detection of Active KHV infection:

PCR test: Detection of KHV during active infection can be done by detecting the viral genome in the infected tissues. Many tissues, such as gills, spleen, kidney and brain, are susceptible to KHV during acute infection, and can be used for KHV genome detection. During acute infection, viruses are shed in the skin and fecal materials, which can also be used for KHV diagnosis.

Virus isolation: Virus isolation is a traditional method used for detection of infectious virion in the infected fish. The best sample for virus isolation is the tissues showing lesions. This method takes several days to perform and is not commonly requested by clients or clinician.

Detection of Latent KHV Infection:

1) **PCR**: During KHV latent infection, only the viral genome is present in some of the lymphocytes. To determine if a koi is a KHV carrier, white blood cells from the peripheral blood can be sampled and tested by PCR using KHV specific primers. Testing WBC for KHV latency is available at Oregon Veterinary Diagnostic Lab (OVDL) via special request.

2) **ELISA**: KHV latent infection can also be determined by detection of KHV specific antibodies using ELISA. If the koi has anti-KHV antibodies in the blood, it will strongly suggest the koi has been exposed to KHV and the koi is a carrier. The ELISA test for KHV is available at UC Davis.

3) **Serum Neutralization (SN assay)**: Similar to ELISA, antibodies produced post-KHV infection can be tested against KHV in the tissue culture. If protection against KHV infection in KF-1 cells is found, it suggests anti-KHV antibodies are present in the blood and the koi is a carrier. The SN for KHV can be requested at UGA and OVDL.

   Note: KHV latent infections cannot be detected by PCR testing of gill snips, vent swabs or fecal examination unless the latent viral DNA has been reactivated and produces infective virions. Detection of the KHV genome in the gill snips, gill swabs or vent swabs suggests an active infection of KHV.

References:

Adkison, M.A., Gilad, O., Hedrick, R.P., 2005. An Enzyme Linked Immunosorbent Assay (ELISA) for Detection of Antibodies to the Koi Herpesvisrus (KHV) in serum of Koi *Cyprinus carpio*. Fish Pathology, 40 (2), 53-62.

Aoki, T., Hirono, I., Kurokawa, K., Fukuda, H., Nahary, R., Eldar, A., Davison, A.J., Waltzek, T.B., Bercovier, H., Hedrick, R.P., 2007. Genome sequences of three koi herpesvirus isolates representing the expanding distribution of an emerging disease threatening koi and common carp worldwide. Journal of virology 81, 5058-5065.

Bondad-Reantaso, M.G., Sunarto, A., Subasinghe, R.P., 2007. Managing the koi herpesvirus disease outbreak in Indonesia and the lessons learned. Developments in biologicals 129, 21-28.

Eide, K., Miller-Morgan, T., Heidel, J., Bildfell, R., Jin, L., 2011a. Results of total DNA measurement in koi tissue by Koi Herpes Virus real-time PCR. Journal of virological methods 172, 81-84.

Eide, K.E., Miller-Morgan, T., Heidel, J.R., Kent, M.L., Bildfell, R.J., Lapatra, S., Watson, G., Jin, L., 2011b. Investigation of koi herpesvirus latency in koi. Journal of virology 85, 4954-4962.

Eide, K.E., Miller-Morgan, T., Heidel, J.R., Kent, M.L., Bildfell, R.J., Lapatra, S., Watson, G., Jin, L., 2011c. Investigation of koi herpesvirus latency in koi. Journal of virology 85, 4954-4962.

Gilad, O., Yun, S., Andree, K.B., Adkison, M.A., Zlotkin, A., Bercovier, H., Eldar, A., Hedrick, R.P., 2002. Initial characteristics of koi herpesvirus and development of a polymerase chain reaction assay to detect the virus in koi, Cyprinus carpio koi. Dis Aquat Organ 48, 101-108.

Grimmett, S.G., Warg, J.V., Getchell, R.G., Johnson, D.J., Bowser, P.R., 2006. An unusual koi herpesvirus associated with a mortality event of common carp Cyprinus carpio in New York State, USA. Journal of wildlife diseases 42, 658-662.
Hedrick, R.P., Groff, J.M., Okihiro, M.S., McDowell, T.S., 1990. Herpesviruses detected in papillomatous skin growths of koi carp (Cyprinus carpio). Journal of wildlife diseases 26, 578-581.
Marcos-Lopez, M., Gale, P., Oidtmann, B.C., Peeler, E.J., 2010. Assessing the impact of climate change on disease emergence in freshwater fish in the United Kingdom. Transboundary and emerging diseases 57, 293-304.
Miyazaki, T., Kuzuya, Y., Yasumoto, S., Yasuda, M., Kobayashi, T., 2008. Histopathological and ultrastructural features of Koi herpesvirus (KHV)-infected carp Cyprinus carpio, and the morphology and morphogenesis of KHV. Diseases of aquatic organisms 80, 1-11.
Omori, R., Adams, B., 2011. Disrupting seasonality to control disease outbreaks: the case of koi herpes virus. Journal of theoretical biology 271, 159-165.
Pikarsky, E., Ronen, A., Abramowitz, J., Levavi-Sivan, B., Hutoran, M., Shapira, Y., Steinitz, M., Perelberg, A., Soffer, D., Kotler, M., 2004. Pathogenesis of acute viral disease induced in fish by carp interstitial nephritis and gill necrosis virus. Journal of virology 78, 9544-9551.
St-Hilaire, S., Beevers, N., Way, K., Le Deuff, R.M., Martin, P., Joiner, C., 2005. Reactivation of koi herpesvirus infections in common carp Cyprinus carpio. Diseases of aquatic organisms 67, 15-23.
Sunarto, A., McColl, K.A., Crane, M.S., Sumiati, T., Hyatt, A.D., Barnes, A.C., Walker, P.J., 2011. Isolation and characterization of koi herpesvirus (KHV) from Indonesia: identification of a new genetic lineage. J Fish Dis 34, 87-101.
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. The Journal of general virology 86, 1659-1667.
Xu, J.R., Bently, J., Beck, L., Reed, A., Miller-Morgan, T., Heidel, J.R., Kent, M.L., Rockey, D.D., Jin, L., 2013. Analysis of koi herpesvirus latency in wild common carp and ornamental koi in Oregon, USA. J Virol Methods 187, 372-379.