

Koi Herpesvirus (KHV) Disease¹

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Introduction

Koi herpesvirus (KHV) is a highly contagious viral disease that may cause significant morbidity and mortality in common carp (*Cyprinus carpio*) (Hedrick et al. 2000; OATA 2001). Common carp is raised as a foodfish in many countries and has also been selectively bred for the ornamental fish industry where it is known as koi. The first recognized case of KHV occurred in England in 1996. Since then other cases have been confirmed in almost all countries that culture koi and/or common carp with the exception of Australia (Hedrick et al. 2000; OATA 2001; Anonymous 2003; Pokorova et al. 2005). This information sheet is intended to inform veterinarians, biologists, fish producers and hobbyists about KHV disease.

What Is KHV?

Koi herpesvirus (also known as cyprinid herpesvirus-3 or CyHV-3) is classified as a DNA virus belonging to the virus family Herpesviridae (i.e., a herpes virus). Although there has been some scientific discussion regarding the accuracy of this classification (Ronen et al. 2003), recent work (Waltzek et al. 2005) shows strong evidence that KHV is indeed a herpes virus, based on morphology and genetics, and is closely related to carp pox virus (cyprinid herpesvirus-1 or CyHV-1) and hematopoietic necrosis herpesvirus of goldfish (cyprinid herpesvirus-2 or CyHV-2). Koi herpesvirus disease has been diagnosed in koi and common carp (Hedrick et al. 2000; OATA 2001). Other related cyprinid species such as the common goldfish (*Carassius auratus*) and grass carp (*Ctenopharyngodon idella*) appear to be clinically unaffected by KHV although KHV DNA has been

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1. This document is Fact Sheet VM-149, one of a series from the School of Forest Resources and Conservation Program in Fisheries and Aquatic Sciences and the Department of Large Animal Clinical Sciences (College of Veterinary Medicine), Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. First published: June 2004. Minor revision June 2004. Major revision October 2008. Please visit the EDIS Web Site at <http://edis.ifas.ufl.edu>.
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identified in tissue of goldfish and other fish species using polymerase chain reaction (PCR) (Bergmann et al. 2006) testing methods. Hybrid goldfish (male goldfish *C. auratus* x female common carp *C. carpio*) were moderately resistant to mortality following experimental infection with KHV (Hedrick et al. 2006). As with other herpes viral infections, KHV is believed to remain in the infected fish for life; therefore, exposed or recovered fish should be considered as carriers of the virus (OATA 2001; Petty and Fraser 2005).

Koi herpesvirus disease may cause between 80-100% mortality in susceptible populations, with clinical signs of disease most commonly being expressed when water temperatures are between 72° and 81°F (22° and 27 °C) (OATA 2001). This viral disease affects fish of various ages, but cohabitation studies show that fry have a greater susceptibility than mature fish (Perelberg et al. 2003). However, larger koi are commonly diagnosed with KHV disease in clinical cases submitted to laboratories.

What Are the Signs of KHV?

Clinical signs of KHV are often non-specific. Mortality may begin very rapidly in infected populations, with deaths starting within 24 to 48 hours after the initial onset of clinical signs. In experimental studies, 82% of fish exposed to the virus at a water temperature of 72°F (22°C) died within the first 15 days (Ronen et al. 2003). KHV infection may produce severe gill lesions which exhibit as gill mottling with red and white patches (Figure 1) (may be similar to columnaris disease signs). The white patches are due to necrosis (death) of the gill tissue. Gill lesions caused by KHV disease are the most common clinical signs in affected koi. Other external signs of KHV may include bleeding gills, sunken eyes, pale patches or blisters on the skin. Some KHV infected koi may have a notched nose (A. Goodwin, University of Arkansas at Pine Bluff, personal communication). In some cases, secondary bacterial and parasitic infections may be the most obvious problem, masking the damage caused by the primary viral infection. Microscopic examination of gill biopsies often reveals high numbers of bacteria and various parasites (Hedrick et al. 2000; OATA 2001; Goodwin 2003).

Internal signs of KHV are variable and non-specific but may include greater than normal adhesions in the body cavity and a mottled appearance of internal organs (Hedrick et al. 2000; Goodwin 2003).

Behaviorally, affected fish often remain near the surface, swim lethargically and may exhibit respiratory distress and uncoordinated swimming.



Figure 1. Koi with mottled gills and sunken eyes due to koi herpesvirus disease. Credits: Deborah B. Pouder, University of Florida

How Do Fish Get Infected With KHV?

Methods of spreading (transmission) of KHV include direct contact with infected fish, fluids from infected fish and water, mud or other fomites/vectors that have come into contact with contaminated systems. The infective virus enters susceptible fish through the gills and possibly through the gut (Dishon et al. 2005). Depending upon water temperature, susceptible fish that are exposed to KHV may either become infected, develop disease, and die or may survive the initial outbreak of the disease and become carriers of the virus (OATA 2001). It is believed that carriers of KHV disease will never again show clinical signs of KHV disease although they are still infected and spread disease to susceptible fish.

Bergman et al. (2006) detected KHV DNA in clinically healthy goldfish and other ornamental fish species, indicating that these species may also carry the virus and may possibly shed virus and cause KHV

disease in susceptible carp. Goldfish carrying KHV do not show signs of KHV disease.

How Does Water Temperature Affect KHV Disease?

The virus appears to have an incubation period of 14 days following the mixing of infected fish or carriers with naïve fish (fish that have not been infected with KHV before) (OATA 2001; Ronen et al. 2003). However, incubation may be longer, indicating that temperature and possibly a second trigger may be necessary for outbreaks to occur. Mortality related to KHV disease typically occurs between 72° and 78°F (22° and 25.5°C). Almost no mortalities occur below this range, and there is virtually no occurrence of the disease at or above 86°F (30°C) (OATA 2001; Goodwin 2003).

How Do I Know if My Fish Have KHV?

Positive diagnosis of KHV requires the assistance of a veterinarian or fish health specialist and a fish disease diagnostic laboratory. Diagnostic identification of KHV may be accomplished by several direct and indirect methods. Direct methods are procedures that detect actual virus or “pieces” of virus. Indirect methods are procedures that quantitate the immune response by measuring antibody levels (Hedrick et al. 2000; OATA 2001; Goodwin 2003).

Direct methods used to identify KHV include: 1) virus isolation and identification (i.e., growing the virus) using a susceptible cell line such as Koi Fin (KF) cell lines {optimal growth observed at temperatures between 59° and 77°F (15° and 25°C)} and 2) PCR techniques (i.e., testing for the presence of KHV DNA material). For these direct diagnostic tests, tissues are removed from fish that are collected alive then euthanized. Isolation and detection of the virus in tissues from fish dead longer than a few hours may be unreliable. Non-lethal direct diagnostic tests are available on samples such as blood, fecal material, mucus and gill clips (i.e., biopsies), but these tests may yield less definitive or less accurate results. A positive cell culture test indicates an active, ongoing infection with KHV.

Positive detection of KHV DNA using PCR indicates that the virus is present, so it identifies koi sick with KHV and may detect some KHV carriers.

Indirect tests for KHV include enzyme-linked immunosorbent assay (ELISA) and virus neutralization (VN) testing. These tests may be performed on a blood sample and, therefore, are non-lethal diagnostic tools. ELISA or VN can provide evidence that a fish has or at one time did have an immune response (i.e., production of antibodies) against KHV. A positive ELISA or VN test for KHV indicates that the fish has produced antibodies against KHV and is either experiencing an outbreak or is a carrier. However, antibody-producing immune cells take time to become activated, and over time, if a fish is no longer sick, KHV-specific-antibody production may slow down or stop. Therefore, ELISA or VN may not be able to detect antibodies to KHV if the infection occurred years before or if the fish has not yet had time to produce antibodies.

Negative results by either direct or indirect tests do not necessarily mean fish are not carriers. There is no test that definitively detects all carriers or survivors.

Is There Treatment for KHV?

There is no treatment for KHV. Antiviral drugs are not available to treat KHV or any other viral diseases of cultured fish. Studies have shown that koi may survive an outbreak of KHV if water temperatures are increased to 86°F (30°C) during the outbreak (Ronen et al. 2003). However, this technique only marginally increases survival rates, and artificially raising water temperatures above 80°F in holding facilities may result in an increased occurrence of other more common bacterial and parasitic diseases. High water temperatures are not generally recommended for routine husbandry and management of koi and common carp. In addition, and more importantly, koi which survive a KHV outbreak or those exposed at high water temperatures will become carriers of the virus. These carrier koi are a source of the disease to susceptible fish when conditions are appropriate for viral shedding and infection. Carrier fish will typically not succumb to KHV disease or show signs of clinical infection.

Currently there is no approved vaccine in the U.S. for KHV. However, preliminary experimental vaccine studies using a live attenuated virus for vaccine by intracoelomic injection demonstrated that fish developed high antibody titers and were immune to the disease and survived a KHV disease challenge (Ronen et al. 2003). However, there is the potential that these fish may be carriers of the virus and may infect naïve fish with which they come into contact, and indirect testing (for antibodies) may be confusing if vaccination status is uncertain.

Because KHV outbreaks have caused large losses at koi and common carp facilities and because there is concern that survivors are carriers, anyone with koi that have been diagnosed with KHV should consider depopulation (eliminating the entire population) as a logical option. All materials and systems that the infected fish have contacted should be cleaned and disinfected.

Viral particles in water may be active in water up to three days (Yoshimizu et al. 2006). However, common disinfection protocols (see below) may be used to eliminate the virus from water systems and equipment [see UF/IFAS fact sheet “Sanitation Practices for Aquaculture Facilities” (VM-87)]. Biofilters and biofilter media exposed to the virus should also be thoroughly cleaned and disinfected. Prior to disinfection, equipment should be cleaned of debris or organic build-up, as these may reduce the effectiveness of the disinfectant. Chlorine solutions (e.g., household bleach) may be used to disinfect large equipment or systems without fish. The recommended protocol for chlorine is 200 ppm (200 mg/L) for one hour (Noga 1996). Proper dosing of this active ingredient depends upon the type of chlorine used. For household bleach, which is 5.25% sodium hypochlorite per liter, 35 milliliters per gallon of water will give 200 mg/L final concentration.

Quaternary ammonium compounds (QACs) may also be used for systems and equipment. Quaternary ammonium compounds are gentler on nets than chlorine solutions. The recommended QAC concentration for disinfection is 500 ppm (500 mg/L) for one hour (Noga 1996). Proper dosing of QACs depends upon the type/concentration in the mixture used because concentrations will vary depending

upon the product used. Different QAC products may range from 10% to 50% active ingredient. For example, Roccal-D Plus® (Pharmacia & Upjohn Company, Pfizer) is approximately 24% active ingredient; therefore, a final treatment concentration of 500 mg/L would require about 7.9 mL of Roccal-D Plus® per gallon of water. Rinse thoroughly after use of any type of disinfectant to eliminate residual disinfectant which may kill fish.

How Can KHV Be Prevented?

Prior to obtaining any fish, first ask the supplier if there have been any major unexplained losses in the population. Monitoring and testing for KHV may be done by laboratory tests, so ask suppliers if any testing for KHV has been done and request a copy of lab result documentation. The best way to prevent KHV is to know your fish suppliers and to have a good working relationship with them.

Quarantine is the most dependable method to avoid the introduction of pathogens into a pond or facility. To implement an effective quarantine procedure, all new fish must be kept in a separate system, ideally in a different building or area from the resident fish. Resident fish should be fed, handled, and maintained before the new fish. The quarantined fish require dedicated equipment such as nets, buckets, and siphon hoses that are used only for them. In addition, foot baths and hand washes should be used by anyone entering and leaving the quarantine area. Fish should be quarantined for a minimum of 30 days. Specifically for KHV, new koi should be quarantined in water that is 75°F (24°C) for at least 30 days. At the conclusion of the quarantine period, any sick fish should be examined by a veterinarian and/or diagnostic lab to rule out KHV or other diseases. If all fish appear healthy, blood samples should be collected from these quarantined fish and submitted for antibody detection using either ELISA or VN methods.

Koi hobbyists are encouraged to promote the use of English-style koi shows, which keep different sources (owners) of koi separated during the show and judging. Additionally, separate nets and equipment should be used by all show participants for their own fish. The Japanese-style of show, in which

koi from different owners are placed together in the same tank, may result in spread of disease among susceptible fish. Regardless of the show style, fish returning from shows should be quarantined (that is, separated from other koi) for a minimum of 30 days and held at 75°F prior to being placed back into the general population. For added security, blood sampling may be helpful. Survivors, known carriers, or fish previously exposed to KHV disease should never be used for show.

At the end of the quarantine period and before placing all fish together, place several new koi with several koi from the established population in a separate area away from the rest of the established population and watch them for signs of disease. This "test" can help determine with a smaller number of fish whether placing the two populations together following quarantine could cause problems. Unfortunately, there are no guarantees.

Who Should I Contact If I Suspect My Fish Have KHV or if I Want More Information?

Commercial fish producers, wholesalers, and retailers in Florida may contact one of the following University of Florida Fish Disease Diagnostic Labs*:

In north Florida:

University of Florida
Department of Fisheries and Aquatic Sciences
Fish Disease Diagnostic Lab
Gainesville, Florida
(352) 392-9617 Ext. 230
<http://fishweb.ifas.ufl.edu>

In south Florida:

University of Florida
Tropical Aquaculture Laboratory
Fish Disease Diagnostic Lab
Ruskin, Florida
(813) 671-5230
<http://tal.ifas.ufl.edu>

*Please note: These labs accept cases from commercial fish producers, wholesalers and retailers only.

Hobbyists and personal koi pond owners (or commercial fish producers, wholesalers, and retailers outside Florida) may search for an aquatic veterinarian or aquatic diagnostic laboratory in their area on the AquaVetMed website (<http://www.aquavetmed.info>).

What Are the Regulatory Considerations Associated With KHV?

As of January 2007, KHV was added to the World Organization for Animal Health (OIE) notifiable disease list for fish. Because of this listing the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) asks that accredited veterinarians and diagnostic laboratories report positive cases of KHV to the area veterinarian-in-charge (AVIC) of the state where the fish originated. However, no client information (e.g., name, address) will be requested. This information will help USDA determine what the prevalence of KHV is in the United States. USDA APHIS has no health requirements specific for KHV for koi or other fish moving interstate or internationally. There is no mandatory depopulation for populations of koi infected with KHV. It is up to the owner of those animals to decide what course to take.

Can Humans Get KHV?

There is no zoonotic concern with KHV. The herpes virus responsible for causing KHV disease in fish will *not* cause disease in humans.

How Does KHV Differ From Other Viral Diseases?

It is important to differentiate KHV from other viruses that may cause disease in carp and koi. Two other important viral diseases recognized in carp are spring viremia of carp (SVC) and carp pox (cyprinid herpesvirus-1 or CyHV-1). These diseases have significantly different management and regulatory implications (Table 1). Both KHV and SVC are now listed as notifiable fish diseases by the OIE. However, within the U.S., SVC is considered a foreign or exotic (not present) fish disease of aquacultured susceptible species and, as such, the

accredited veterinarian or laboratory is required to notify confirmed outbreaks to USDA APHIS Veterinary Services officials who will, in turn, notify the OIE. By comparison, KHV is considered to be endemic (continuously present) in the United States. USDA APHIS asks accredited veterinarians and laboratories to report cases of KHV to the area veterinarian-in-charge (AVIC) of the state where the outbreak is occurring. The information will help USDA determine what the true prevalence of KHV is in the U.S. However, every outbreak of KHV will not be notified to the OIE.

Spring viremia of carp disease is caused by an RNA virus, *Rhabdovirus carpio*, and has been reported in common carp and koi (*C. carpio*), grass carp (*C. idella*), bighead carp (*Aristichthys nobilis*), silver carp (*Hypophthalmichthys molitrix*), Crucian carp (*Carassius carassius*) and common goldfish (*C. auratus*). For more information on SVC see Table 1 and the UF/IFAS fact sheet “Spring Viremia of Carp” (VM-142).

Carp pox disease (cyprinid herpesvirus-1 or CyHV-1) is caused by a different herpesvirus (*Herpesvirus cyprini*) that has wide geographic distribution and affects common carp and koi. Carp pox typically causes smooth raised growths (“wart-like masses”) on skin and fins of older fish but may be associated with high mortality in fry less than two months of age (Hedrick et al. 2000). Carp pox is not a reportable or notifiable disease in the U.S. Water temperatures above 68° F (20°C) help reduce the skin and fin growths on older fish, but does not eliminate the virus from the fish (Table 1). In mature fish, carp pox is typically a non-lethal, self-limiting disease (i.e., most if not all growths will resolve at warmer temperatures on their own).

Summary

Koi herpesvirus is a devastating disease of ornamental koi and common carp. There are several methods to detect various states of the infection, but at the current time there are no legally available vaccines in the United States. There is no effective treatment to rid the fish of the virus. Good management practices, including quarantine, testing, and appropriate husbandry, are vital components to

preventing this disease for all koi and common carp producers, dealers, and hobbyists.

Glossary

Adhesions: areas of internal tissues that stick together; may be normal in some species and a result of inflammation in others

Ascites: dropsy; a build up of fluid in the body cavity

Asymptomatic: showing no signs of disease or infection

Attenuated: weakened; less pathogenic/disease-causing

AVIC: USDA APHIS area veterinarian-in-charge; there is one AVIC per state.

Carrier: an animal which harbors a disease-causing agent in its body without showing clinical signs of infection (i.e., they appear healthy but are capable of spreading disease under the right conditions)

Clinical signs: the observation of physical or behavioral abnormalities

CyHV-1: cyprinid herpesvirus-1; the virus that causes carp pox

CyHV-2: cyprinid herpesvirus-2; the virus that causes hematopoietic necrosis herpesvirus of goldfish

CyHV-3: cyprinid herpesvirus-3; the virus that causes koi herpesvirus

Common carp: *Cyprinus carpio*; common carp are the original species of fish from which koi were developed for ornamental purposes

Cyprinid herpesvirus-1: CyHV-1; the virus that causes carp/koi pox

Cyprinid herpesvirus-2: CyHV-2; the virus that causes hematopoietic necrosis herpesvirus of goldfish

Cyprinid herpesvirus-3: CyHV-3; the virus that causes koi herpesvirus

DNA: *deoxyribonucleic acid*; found in a cell's nucleus and is the basic structure of genes

Edema: a build up of fluids within tissues, cells, or body cavities (ascites is a build up of fluid primarily in the body cavity)

ELISA: *enzyme-linked immunosorbent assay*; a lab method used to detect the presence of antibody to a virus

Fomite: an inanimate object or material (e.g., net, siphon hose) on which disease-causing agents may be spread

Gene: unit of heredity consisting of segments of DNA

Intracoelomic: within the body cavity

KHV: koi herpesvirus; the name of the virus that causes koi herpesvirus disease

Koi: a common carp that has been selectively bred for ornamental purposes

Morbidity: disease or sickness

Naïve fish: a fish that has not been exposed or infected by a pathogen

Necrosis: death of tissue

Necrotic: dead tissue

Notifiable disease: a disease which must, by law, be reported to the proper federal (USDA APHIS Veterinary Services) and state (state veterinarian) officials when positively diagnosed

OATA: *Ornamental Aquatic Trade Association*; a British organization representing the interests of ornamental fish importers, breeders, wholesalers, retailers, and manufacturers

PCR: *polymerase chain reaction*; an advanced biochemical test used to identify the presence of specific pieces of an organism's genes (DNA)

Reportable disease: same as notifiable disease; a disease which must, by law, be reported to the proper federal (USDA APHIS Veterinary Services)

and state (state veterinarian) officials when positively diagnosed

Self-limiting: does not cause major disease and usually heals or resolves on its own

SVC: spring viremia of carp

Transmission: the transfer of a pathogen from one animal to another

USDA APHIS: *United States Department of Agriculture, Animal and Plant Health Inspection Service*

Vector: a carrier (e.g., argulus) which transfers an infective agent from one animal to another

Virus neutralization (VN): a lab method used to quantitate antibody to a specific viral pathogen

Zoonotic: a disease of animals (e.g., fish) that can be spread to, and cause disease in, humans

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Table 1. Comparison of koi herpesvirus (KHV), spring viremia of carp (SVC), and carp pox.

	Koi Herpesvirus	Spring Viremia of Carp	Carp Pox
Synonyms	Cyprinid herpesvirus-3 (CyHV-3); carp nephritis and gill necrosis virus (CNGV)	Infectious dropsy of carp	Cyprinid herpesvirus-1 (CyHV-1); koi pox; carp herpes virus; herpesviral epidermal proliferation in carp (HEPC); herpesvirus septicemia in carp (HSC)
Abbreviation	KHV; CyHV-3	SVC; SVCV	CHV; CyHV-1
Viral Agent	Herpesvirus (DNA virus)	Rhabdovirus <i>Rhabdovirus carpio</i> (RNA virus)	Herpesvirus <i>Herpesvirus cyprini</i> (DNA virus)
Species Affected	Common carp; koi; other species may carry virus	Common carp; koi; goldfish; grass carp; bighead carp; silver carp; and Crucian carp	Common carp; koi
Optimal Water Temperature	64-81°F (18-27°C)	41-64°F (5-18°C)	<68°F (<20°C)
Transmission	Direct contact; fecal material; infected water/mud; equipment; vectors	Direct contact; fecal material; infected water/mud; equipment; vectors	Direct contact; fecal material; infected water/mud; equipment; vectors
Age Susceptibility	Young more susceptible than mature	Young more susceptible than mature	Young more susceptible than mature
Clinical Signs			
Behavioral	Lethargy; swim close to the surface; respiratory distress; erratic behavior	Lethargy; low on tank or pond bottom; awkward swimming	None
External	Gill necrosis; sunken eyes; notched nose; secondary bacterial and parasitic infections	Exophthalmia; pinpoint skin hemorrhage; abdominal distention; mucus from vent	Smooth raised wart-like skin lesions
Internal	Few, variable signs	Edema; inflammation; pinpoint hemorrhages of many organs including swim bladder	None
Testing Methods	Direct methods (virus isolation and PCR); indirect methods (ELISA and VN)	Direct methods (virus isolation and PCR)	Direct methods (virus isolation)
Carrier States	Yes	Yes	Yes
Regulatory Status	NOTIFIABLE with no mandatory consequences	NOTIFIABLE with mandatory consequences; import regulations	None
Treatment	None	None	None
Prevention/ Control	Depopulate infected stocks; practice good biosecurity including quarantine and testing; purchase fish from known reputable source; keep susceptible species separated	Depopulate infected stocks; practice good biosecurity including quarantine; purchase fish from known reputable source; keep susceptible species separated	Depopulation generally not required for older fish; practice good biosecurity including quarantine; purchase fish from known reputable source
Disinfection	Chlorine (200 ppm for 1 hour); quaternary ammonium compounds (500 ppm for 1 hour)	Chlorine (500 ppm for 10 minutes); ozone; gamma/UV radiation; pH <4.0 or >10.0; heat 60°C for 15 min	Chlorine (200 ppm for 1 hour); quaternary ammonium compounds (500 ppm for 1 hour)