Koi herpesvirus (KHV)

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Summary and Introduction

KHV is a virus that causes disease in koi and common carp only, is highly contagious, can kill most of the koi in an infected system and has spread world-wide.

Survivors of infections can be carriers.

Hobbyists and dealers can help protect themselves by:

1. Buying from reputable sources that adequately quarantine all fish arriving at their facilities and operate under Best Health Management Practices,
2. Adequately quarantining new fish (and fish returning from outside the facility) prior to (re)introducing them into the general fish populations. **This is the single most effective action within the fish owners' control,**
3. Disinfecting, or otherwise verify the safety (non-infectivity), of everything that comes in contact with water of existing pond/system or new fish,
4. Depopulating all koi and carp exposed to KHV,
5. Supporting efforts to educate the koi community and to find new ways to control and ultimately eradicate this disease, e.g., donating to Project KHV (shameless plug), and
6. Buying vaccinated koi if and when proven safe (and effective) and having existing fish vaccinated.

Testing for antibodies to KHV (an indication of past exposure or vaccination) and for the virus (during active disease) can now be done without killing the fish.

Starting in the Spring of 2007, a course with more current and complete information and presented by very competent instructors will be made available through the sponsorship of AKCA's Project KHV (see announcements in July/Aug '06 and Sept/Oct '06 issues of KOI USA). This fact sheet was assembled to help bridge the gap between then and now.

History

Archived histological material taken from U.K outbreak in 1996 was later tested and KHV DNA was shown to be present in these samples (Way *et al.*, 2004)

First found in Israel in May 1998 (Hedrick, *et al.*, 1999)

First found in the U.S. in fish from the East Coast in August, 1998 (Hedrick *et al.*, 2000)

Virus first isolated and partially characterized in 2000 (Hedrick *et al.*, 2000)

First PCR tests developed in 2002, Gilad and Gray produced different primers (Gilad *et al.*, 2002 and Gray *et al.*, 2002)

Has been found in most countries in the world where koi and/or carp are commonly raised or kept – (Pokorova, *et al.*, 2005)

What is Known?

Virus and disease characteristics:

Is a herpes-type virus the genome of which is a double stranded DNA molecule of approximately 295 kilo base pairs (Haenen *et al.*, 2006 – Aoki presentation). KHV is relative large as compared to other known mammal and bird herpesviruses (125

to 245 kbp), however, CyHV-1 (carp pox) has been estimated to also be about 295 kbp. KHV is proposed as a third cyprinid herpesvirus (CyHV-3) in the family *Herpesviridae.* (Waltzek *et al.,* 2005)

Others resist the classification as a herpesvirus and prefer the designation CNGV. (Dishon, *et al.*, 2005)

Is highly contagious and can produce high (80% to nearly 100%) mortality in diseased populations of koi and common carp (Dishon *et al.*, 2005)

Appears to cause disease and mortalities only in common carp and koi but the virus can also infect goldfish and crucian carp (Haenen *et al.*, 2006 –Bergmann & Tinman presentations)

Infections are transmitted via virus in the water, in fecal material, in sediments and from fish to fish (Dishon *et al.*, 2005; Hartman, *et al.* 2004)

The route of infection into the fish is likely thru the gills (Dishon *et al.* 2005) and the gut (Haenen *et al.*, 2006 – Bergmann presentation)

Propagates mainly in intestine and kidney of infected fish (Dishon *et al.*, 2005)

Deaths can start within 1 to 2 days following the onset of clinical signs (Hartman *et al.*, 2004)

Infected fish usually die within 6 to 24 days (post infection) at permissive temperatures (Dishon *et al.*, 2005)

Permissive temperatures are variously reported as 17-26°C; 18-27°C; 18-25°C; 22-26° C and 18-28°C (Haenen *et al.*, 2004; Hartman *et al., 2004;* Ronen, *et al.*, 2003; Perelberg *et al.*, 2003 & Haimi, 2003 [plus several others], respectively)

Temperature ranges for optimum virus growth in cell cultures tend to be 2-3° C wider than those in fish (Gilad *et al.*, 2003).

Can produce latency and/or a persistent low-level infection such that survivors can later become infectious (St-Hilaire *et al.,* 2005)

Can survive off the fish for weeks, probably in sediments and/or filters (Haenen *et al.*, 2006 –Bergmann presentations)

Survives in water for 4 hours but less than 18 hours (Perelberg *et al.*, 2003)

Clinical signs seem to be arrested at >30° C and fish raised to this temperature can often survive the disease (Ronen *et al.*, 2003)

Clinical signs seem to be arrested at <13° C (Hedrick *et al.*, 2005)

Does not appear to cause disease at or below 13° C (55° F) – (Gilad *et al.*, 2003)

Antibodies have been found in survivors up to a year post infection (Haenen *et al.*, 2006 –Dixon presentation)

Virus found up to 7 months post infection in the base of the gills, the kidney, the spleen and the leukocytes (Haenen *et al.*, 2006 – Bergmann presentation)

The entire viral genome has been sequenced (Haenen *et al.*, 2006 – Aoki presentation)

There are several different strains (mutations) of the virus (Haenen *et al.*, 2006 – Ito presentation)

There is no zoonotic concern with KHV. (Hartman *et al.*, 2004; Southard *et al.*, 2006)

Disease symptoms: (Hartman, *et al.*, 2004; Goodwin, 2003; Groff, 1999- pers. comm.)

Not all diseased fish exhibit all symptoms

Pale and necrotic gills (gill rot) is the most common symptom – see Figure 1

Hyperplastic gills observed with or w/o bacterial infection (gill rot)

Lethargy and "hanging" in the water toward the end-stage of the disease

Sunken eyes

Erratic swimming

A notch in the "nose" of the fish – see Figure 2

Figure 1. Necrotic gills
(Photo by Duncan Griffiths)

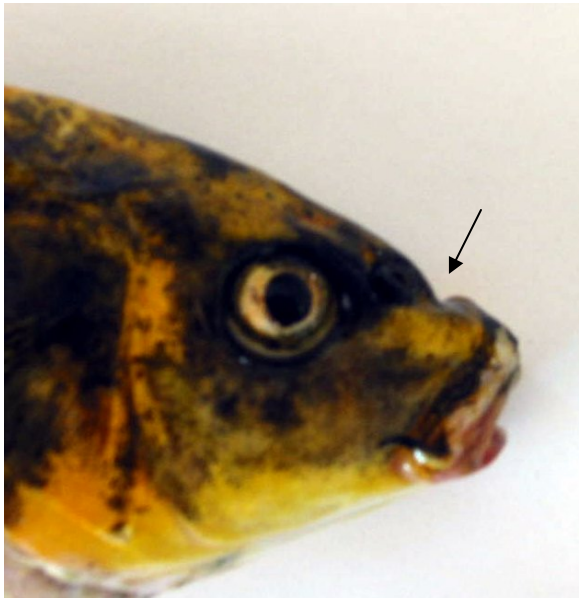

Figure 2. Notch in "nose" (see arrow)
(Photo by Jeff Nicholson)

Heavy mucus at the start of disease

Lack of mucus later as disease progresses – skin feels like sandpaper

Patches of discolored skin

Group contains symptomatic koi and/or carp but other species show no symptoms

Testing – availability and tissues for sampling (partial listing):

KHV typically found in the kidneys first and longest over the course of the active disease so good tissue to test

Gills, spleen, fins and gut are also good tissue to test if the disease is suspected

PCR tests for viral DNA – commercially available (fee-for-service) in the US (TX, GA, FL, WA), Japan, UK, Germany, Israel as well as other countries

Serology tests for antibodies – commercially available in US (GA), UK (CEFAS), Israel

Histopathology – commercially available in US (GA)

Virus Isolation (in cell culture) – commercially available in US (GA)

ELISA tests for virus – not currently commercially available in US

ELISA test for antibodies to KHV – in US (UC Davis) and commercially in UK (CEFAS) and Israel

LAMP test for DNA - not currently commercially available in US, avail in UK

Tissue samples (e.g., gill, kidney, etc.) may be placed in rubbing alcohol (70% or 90%) for later PCR testing – (Goodwin, 2005- pers. comm.)

Droppings of infected fish have been shown to contain KHV that can be detected by PCR testing (Dishon, *et al.*, 2005)

Cloacal swabs may be submitted to some labs for PCR testing (KHV DNA) – Dacron-tipped swabs are preferred over cotton-tipped swabs (Q-tips), (Colaizzi, 2006- pers. comm.)

What is Unknown?

If and where the virus exists for periods >1 year inside the fish when it's not causing disease

If and where the virus exists for long periods when in the environment (outside the fish)

Testing that can determine if a specific fish carries the (internal) potential to become diseased or infectious (even if it "passes" quarantine), i.e., a test to determine latency

If there is a safe and effective way to rid latent or persistently infected fish of the virus or the potential to become infective

If there is a vaccine possible that will confer long term protection against KHV

An effective field test for KHV

An effective field test for KHV antibodies

If there is vertical transmission

Control and Prevention

Purchase decision:

Buy koi from reputable sources that adequately quarantine new fish arriving in their facility and operate under Best Health Management Practices.

Quarantine:

Quarantine all new fish, and those returning to the facility, at 70° F to 75° F for at least three weeks.

Adding SPF (KHV naïve) fish to the quarantine group is probably helpful as naïve fish are more likely so show disease symptoms (Haenen *et al.*, 2004)

Observe fish in quarantine system noting symptoms and behaviors, particularly those typical of KHV – see "Disease symptoms" in this fact sheet.

Use separate equipment to house and handle quarantined fish.

Thoroughly disinfect equipment if it is used between systems and/or between separate lots of fish.

Serology or antibody testing during quarantine can determine if fish have been exposed to KHV and/or a KHV simulating antigen (as in a vaccine) and have developed antibodies to the virus. This could identify potential carrier fish or it could simply indicate that a fish has been successfully vaccinated. Knowing the fish's history would be helpful in this event. Having a "marker" in the vaccine would be even better.

Disinfection of holding facilities and fish handling equipment may be accomplished using:

a. Household bleach, a sodium hypochlorite solution (usually 5.25%). Good disinfectant, particularly for ponds and for infrequent or one time disinfecting of large equipment. Recommended concentrations and exposure times are 200 ppm (~35 ml bleach per gallon of water) for one hour (Noga, 1996; Herwig, 1979) or 10 ppm (~1750 ml or ~ ½ gal. bleach per 1000 gallons water) for 24 hours (Herwig, 1979)
   Note: Bleach is toxic to fish and harmful to metal and netting but can be neutralized with sodium thiosulfate pentahydrate ("ST" – the active ingredient in dechlor). Use 7.4 mg of ST to neutralize 1 mg of chlorine (Saint-Erne, 2002); ~5600 grams or 12.4 pounds of ST per 1,000 gallons of water to neutralize sodium hypochlorite at 200 ppm.
b. Quaternary ammonia compounds at 2000 mg/l for one minute (Billard, 1995) – net dip
c. Calcium hypochlorite at 180-200 mg/l for 20 seconds (Billard, 1995)

Disinfection of hands may be accomplished by (Billard, 1995):

a. Quaternary ammonia compounds at 1000 mg/l
b. Iodophor at 100-200 mg/l

Disinfection of clean boots may be accomplished by (Billard, 1995):

a. Quaternary ammonia compounds at 2000 ppm for one minute.
b. Calcium hypochlorite at 120 mg/l of chlorine for 20 seconds
c. Sodium Hypochlorite at 180 mg/l of chlorine for 20 seconds

Disinfection can be compromised (incomplete) if items are contaminated with debris and/or have rough or porous surfaces. Clean items prior to disinfection and increase the exposure time for rough and/or porous items.

Care should be taken that disinfecting solutions are clean and active. Replace as necessary.

Depopulation:

If KHV is confirmed, depopulation (killing all the infected and exposed fish) should be considered as carriers may exist in any survivors.

Vaccination:

There are currently 6 groups reported to be working on vaccines for KHV. Immunizing "agents" are reported to include killed-virus, attenuated-live virus and DNA. Delivery techniques include oral, immersion and injectable. The groups include:

- Yoshimura & Miyazaki, Mie University, Japan,
- Aoki, Tokyo University, Japan,
- Perelberg & Kotler, Hebrew University-Hadassah Medical School, Israel,
- Shivappa & Levine, North Carolina State University, CVM, USA,
- Ritchie, University of Georgia, USA, and
- Novartis, Canada

Online Resources

Veterinarians

http://www.aquavets.com/index.cfm
http://www.akca.org/kht/vet_refer.htm
http://www.fishdoc.net/vet/index.php

Laboratories

http://www.aquavets.com/index.cfm
http://www.koilab.com/
http://www.vetdna.com

Koi Health Advisors

http://www.akca.org/kht/graduate.htm

Glossary

AKCA - Associated Koi Clubs of America, an organization of koi clubs within the U.S. and Canada

Antibodies – proteins produced by the immune system to fight specific bacteria, viruses, or other antigens.

Antigen - substance capable of inducing an immune response.

Base pairs (relative to DNA) - Pairs of complementary nitrogenous bases that interact to form the rungs of DNA's double helix "ladder" structure. Adenine (A) pairs with thymine (T); cytosine (C) pairs with guanine (G).

Benzalkonium chloride (alkyl dimethyl benzyl ammonium chloride) - an organic compound used as a disinfectant.

Cloaca - an organ into which an animal's digestive, urinary and reproductive systems empty, and that opens to the anus.

CNGV - carp interstitial nephritis and gill necrosis virus, a designation for the virus preferred by some in the scientific community.

Disease - an impairment of health or a condition of abnormal functioning.

Disinfection - the effective elimination of disease causing organisms.
DNA - deoxyribonucleic acid, the material inside the nucleus of cells that carries genetic information.
ELISA - Enzyme-Linked Immunosorbent Assay, a widely used immunochemical method for detecting antigens or antibodies. ELISA methods are carried out in microtitre plates and use colorimetric detection.
Genome - the complete set of genetic information of an organism including DNA and RNA.
Histology - the study of cells and tissue on the microscopic level.
Hyperplasia - an increase in the number of cells in a tissue or organ, excluding tumor formation.
Infect – to invade with a foreign organism, typically a pathogen.
Iodophor - a substance consisting of iodine and a solubilizing agent that releases free iodine when in solution.
Kilo base pairs - thousands of base pairs
Koi Health Advisor (KHA) - a koi hobbyists that has completed the training and continuing education required by the AKCA's KHA Program.
KHV - koi herpesvirus, the virus that causes KHV disease.
LAMP - Loop-mediated isothermal amplification is a test for DNA.
Latency (with regard to viral infections) - a dormant disease that can later become infective.
PCR - polymerase chain reaction; a technique for amplifying DNA, making it easier to isolate, clone and sequence.
Permissive temperatures – temperatures at which KHV disease is typically observed in fish.
Project KHV – an AKCA committee formed to solicit funding to support KHV research and education. see:http://www.akcaprojectkhv.org/index.htm
Quarantine: forced isolation
Quaternary ammonia compounds – disinfectants (e.g., benzalkonium chloride and Roccal®)
SPF - specific pathogen free – in this context, meaning free of KHV infection or exposure, i.e., naïve to KHV
Serology – the branch of science dealing with the measurement and characterization of antibodies and other immunological substances in body fluids, particularly serum.
Serum – The clear liquid part of the blood that remains after blood cells and clotting proteins have been removed.
Vertical transmission - disease passed from parent to offspring through eggs or sperm.
Virus - a small particle that infects cells in biological organisms. Viruses are obligate intracellular parasites; they can reproduce only by invading and taking over other cells as they lack the cellular machinery for self reproduction.
Zoonosis - An infection or infestation shared in nature by humans and other animals that are the normal or usual host; a disease of humans acquired from an animal source.

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