Do You Really Know KHV?
By Mathew Vangel

Background-
You have probably read numerous articles about the Koi Herpes Virus (KHV). I have reviewed many of these research papers and found simply that the same information was written over and over again. Recently in New England, an outbreak of KHV has demonstrated many new undocumented behavioral attributes. Some of these new symptoms might be attributed to secondary complications of the disease. The information in this article is meant to increase your awareness, describe observed symptoms of this disease, and assist you in the protection of your koi. Topics that are covered include the following:

- Longevity of the active virus
- Best practices for virus detection
- Treatment procedures that may reduce your KHV fatalities

This content is presented in a general audience format. A more formalized technical essay will be completed after this summer's (2006) research is done. The details in this article have been collected from 3-months of monitoring and treating validated virus outbreaks with KHV carriers (imported koi). Each site tested positive for KHV using PCR (Polymerase Chain Reaction) testing, which was sent to the University of Arkansas. These sites vary in type from nurseries, home hobbyists, and general fish retailers who experienced over 1,800 koi deaths. These deaths were attributed to the disease, and did not result from parasites. This was confirmed by extensive microscopic analysis at the time of death. Due to misfortune, our own test pond's koi enabled additional research data for this article, so that we may transfer this knowledge to you.

Before I start describing our "hands-on" KHV experiences, I am required to give you background characteristics of the testing facilities environment- pH-7.2, Dissolved Oxygen 8ppm; Average water temperature 76°F, and salinity- 3.0ppt or .3%. Commercial UV sanitizers-digitally monitored with low-flow configurations, and commercial bead filtration system. General water conditions monitored 7x24 by electronic data loggers.

Point to remember- a virus does not have to behave exactly as you expect. It can easily evolve into something quite different although still maintaining the same end result...death.

What does the disease look like?
The KHV virus is similar to a cold virus where symptoms can vary widely from person to person, or koi to koi. Some fish exhibit the common documented symptoms of sunken eyes (shown in **Figure-1**), scale loss due to epidermis decay, bare epidermis patches (shown in **Figure-2**), and a reduction of slime coat production (entire body of koi feels like sandpaper).

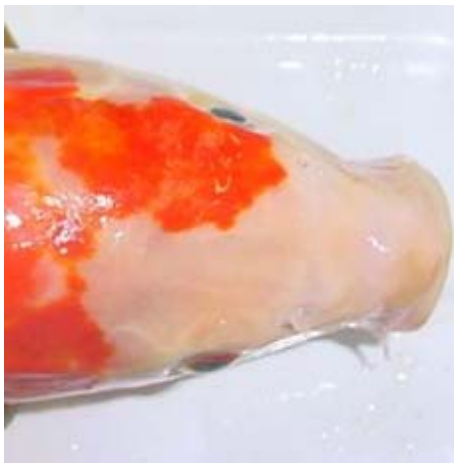

Figure-1 Deceased koi with sunken eyes

Figure-2 Bare patches on sides of koi

Recently observed symptoms have been documented from the New England KHV outbreak. They include:

- Blindness
- Shallowing of the body starting at the pectoral fins to the Caudal fins, where the fish becomes a swimming skeleton
- Loss of body fluids causing "dehydration"
- Fading of pigment color
- Uncontrolled shivering
- Intermittent violent shaking of the head
- Spiral swimming
- Neurological damage
- Unbalanced swimming- The koi will constantly tip left and right.
- Using pectoral fins for forward movement by "dog paddling" instead of using the Caudal fin

Secondary effects of the virus occur once the immune system is compromised. They include the following:

- Parasite infestation
- Bacterial infections (as shown below in **Figure-3**)

Figure-3 Bacterial infection

Real Case Study- Boston, MA. Area Nursery

How do you think KHV is spread to koi who have never been exposed to the virus (Naïve)?

One of my customers has a garden center, which was the first location in New England to be devastated by KHV this summer. This customer has 8 fish tanks using a common filter system and water distribution system. KHV carriers were received from their fish supplier and placed in tank #3 of their 8 tank retail display setup. All 8 tanks are connected to each other in a closed loop system utilizing the same filtration process. These tanks are enclosed in a greenhouse where the average ambient

daytime temperature is 84°F. Each of the fish tanks held between 20-75 koi from previous stock purchases. The first koi death was noticed 5-days from the date of exposure to the KHV carriers. The number of deaths were 20-35 per/day at the peak of the KHV outbreak. The fish deaths were limited to tanks 3, 4, and 5 while koi residing in holding tanks 1, 2, 6, 7, and 8 remained healthy, and were never affected.
It is suggested that the virus was distributed by the use of a fish net. Customers would regularly ask the nursery to net fish so they could make a buying decision. Those fish deemed to be unsatisfactory were returned to the tank. This same net was also used to remove each of the fish that died due to KHV, and was only handling fish from tanks 3, 4, and 5. The KHV virus did not propagate into the other tanks even with a shared water filtration system. Because koi are very social and communicate by touch and posture, the virus was easily spread in each of the tanks where an infected host resided.

I became involved with this first outbreak when the fish supplier employed me to perform microscopic analysis and collect data at the site due to an extraordinary amount of deaths that were occurring daily.

In order to validate the hypothesis that KHV may only be spread by contact, I initiated a controlled functional test for KHV transmission involving 2-pairs of test subjects purchased at a local pet shop. One pair of koi was placed in a common water source (Non-Contact Subjects) to the KHV carrier fish, and the other pair was placed directly in (Contact Subjects) the same tank with the carriers. From the recent outbreaks in the Northeast, it seems that KHV was transmitted through contact, and not water particulate.

Water temperature of holding tank was maintained at 76°F with a thermostatically controlled heater.

Table-1 shows test group observations

Test Days	**Contact Subjects**	**Non-Contact Subjects**
1-5	No anomalies	No anomalies
6-10	No anomalies	No anomalies
11-15	Unbalanced swimming	No anomalies
16-25	Floating on surface of water	No anomalies

Table-1

Results- The collection of data suggests that KHV demonstrated a higher potential of infection through surface contamination than virus particulate being transported by the water medium.

More studies are needed to validate these finding under a wider variety of environmental conditions.

Quarantine Protocol for KHV?
I have read so many articles about raising temperatures, watching the fish for 20-30-45 days, and even saying the Koi Herpes Virus will usually end in 30-days. I haven't seen any of these pontifications to be true.

The carriers are usually the most healthy individuals, and do not exhibit any indications of health issues.

Shown below in **Figure-4** is one of the 6-KHV carriers currently being held at our location for research purposes.

Figure-4 KHV Carrier

The Koi Herpes Virus has demonstrated a "rapid propagation stage" in some fish, which exhibit no evident internal or external anomalies. Death can occur within 5-days from the first day of exposure with a KHV carrier. This has been recorded with water temperatures of a constant 76°F. Shown below in **Figure-5** is an example of a koi that died from this rapid progression stage of the virus. The gills have been photographed to show that no breakdown of tissue has occurred. Notice the eyes are still extruding from the body of the fish, and have not characteristically receded. The koi died before the virus could sufficiently disrupt its body fluid levels.

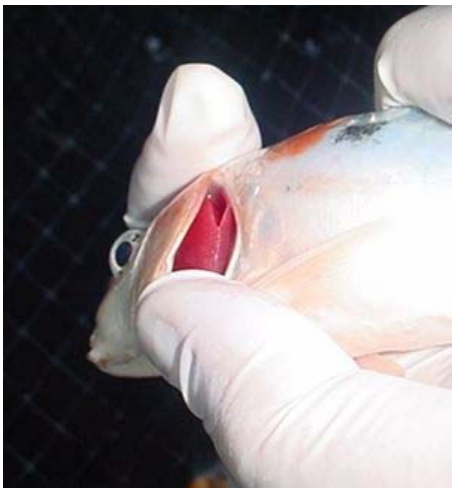

Figure-5 KHV fatality with healthy gills

Fish fatalities were recorded with water temperatures of 86°F lasting a period of 5-days. Even the efficacy period for the virus has far surpassed the recently published activity time frames of 10-30 days. Currently KHV has been effectively killing koi in our test pond for over 60-days since the introduction of the KHV carriers. This demonstrates the virus's longevity. The KHV outbreak has been in remission since the KHV carriers were removed.

What should I do with my new fish?
The following practices should be employed when your fish supplier cannot provide the historical health record for your new fish.

Establish a Baseline-

1. Record the condition of all fish being received. Observe the healthiest fish and isolate these fish from the group when any health issues arise.
2. Never mix fish together that have not had long-term exposure to each other. Keep groups separated maintaining similar water quality parameters.

3. The number of days to quarantine is not a relative metric when KHV could be present. Raising the temperatures of holding tanks could possibly jeopardize your opportunity to immediately identify KHV carriers.
4. Add salt to the holding vessels as the virus interferes with the osmotic process of the fish. You want to maximize fluid retention in each fish. The disease causes koi to lose body fluids.

You can hold KHV carrier koi for **40-years in quarantine** and still not see KHV. My research shows the main asymptomatic carriers were found to be 6 butterfly koi that were imported by the U.S. fish supplier from Vietnam.

Determining the health of new fish before they are sold or released into your own pond is your most important decision point.

There are two approaches that I see as viable methods to detect KHV.

1. Obtain 2-3 test subjects that are domestically grown koi from local pet stores. Observe multiple tanks from the retailer for excessive deaths in any of their koi tanks before you make these purchases.

2. Obtain a tissue sample from the weakest fish. Provide your testing facility with a gill arch dehydrated in rubbing alcohol, or a near-death sample in ice packs.

You are simply following the same approach commonly used in the medical field. When you cannot obtain qualitative health data from your fish hatchery, order lab tests. The U.S. fish supplier responsible for the recent New England KHV outbreak did not test the imported koi. These are the fish which proved to be KHV carriers. Koi fatalities for this one outbreak resulted in 320 koi deaths in New England from the small number of KHV carriers that were sold.

I personally sent out two KHV samples for a Polymerase Chain Reaction (PCR) test to a well-known U.S. location resulting in a false-negative result. Knowing the symptoms resembled KHV, a second laboratory submittal was sent to the University of Arkansas, which resulted in a positive detection for KHV. Be careful where your KHV samples are tested, and don't hesitate to question the results. Ask how many positive results have occurred at their location, and inquire about the reported symptoms associated with the sample that tested positive.

How to Beat KHV……..…
You will have to make a serious decision about being a long-term steward for all of the KHV survivors before you follow any of the following practices to dramatically reduce your losses.

Once the virus has been positively diagnosed, the following procedures will reduce your mortality rate to small percentages:

- pH- 7.2
- Dissolved Oxygen- >7ppm
- Salinity- .3% or 3.0ppt
- Temperature- 20-26C
- Ammonia, Nitrites, Nitrates- 0
- Food- 20-days of triple Antibiotic (e.g. Black Water Creek)
- Parasite Treatment- Formalin type of medication 3-times per week. (e.g. Proform-C)

- Microscopic inspection to verify parasite free environment. Treat water and fish as needed
- Treat secondary infections as needed
- Commercial UV Light- reconfigured as a sterilizer with the water flow rate adjusted from 1000gph (normal rate) to 500gph. A Water-flow manifold must be used to adjust UV exposure rate. Shown below in **Figure-6** is one of our custom-made manifolds.

Figure-6 Digital Water-Flow Adjustment Manifold

- Identify KHV carriers and separate from the general population.

Now what about transient carriers?
It seems many local koi hobbyists favor releasing their sick fish into local streams and ponds. You don't want to transfer any plants, water systems, nets, fish, or other items that have come in contact with the pond water, or have established Biofilm structures on their surfaces (refer to KOI USA article Biofilm July/August 2003 mvangel). Even frogs and turtles have the potential to migrate this virus to other water systems.

Cleaning up after KHV-
After any disease outbreak, the disinfection of systems needs to utilize current solution formulations. Recently recommended disinfection procedures published for the koi industry were developed in the 1940's. These practices are outdated and have matured in most modern medical facilities utilizing improved disinfectants. The Spaulding Disinfection Scale clearly states the level of the virucidal required for this type of application. Always use a pre-measured "ready-to-go" intermediate level disinfection solution. There are non-toxic products to the environment and fish. The use of common bleach can be misapplied, incorrectly mixed, and the residual effects to metal parts and proteinous material makes this solution less than ideal. Please E-mail me with your application for an appropriate "hospital-grade" product recommendation.

Wear appropriate PSE (Personal Safety Equipment) when performing any cleaning procedure, or working with contaminated materials. So many times I see infected fish being handled by people not wearing gloves. Surface contamination is the greatest vehicle for microorganisms to move around.

Further testing- Currently I am discussing the virus with the Massachusetts Fish and Game Division regarding testing regional fresh water game fish with KHV carriers to validate previous hypothesis claims that KHV does not favor species transfer.

Summary
KHV is a serious issue that can devastate our hobby and koi businesses without the implementation of proper policies and procedures. Monitor your current fish anytime new additions are added to your pond, and isolate items that come into contact with your new fish. Don't get anxious to add the recently purchased koi without a complete follow-through of the steps described in this article. Monitor your water conditions throughout the day, and immediately treat any health issues that arise during the KHV outbreak.
If your pond becomes infected with this virus you must be prepared to decide what the future will be for your fish. You may be agreeing to provide long-term care for the survivors of KHV with ancillary disabilities from the virus.

A detailed research white paper will be available in the next few months at my website. Additional KHV symptom movie clips are located at- http://www.koiguru.com/khv

My follow-up article will describe the "Best Practices of Disinfection" that you should follow when handling, and cleaning surfaces relating to your hobby.

Author's Background- Mathew Vangel is an Advanced Technology Research Engineer, and licensed Medical Researcher in Massachusetts studying Infectious Disease Control, cross-contamination from aquatic to human environments, blood pathogens, Biofilm Structures, and Endocrinology. Owner-Koi Pond Services of New England providing on-site medical, maintenance, and mechanical services. For more information and additional article references write to- Koi@KoiGuru.com
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