

What's happening in KHV research?

In 2005 scientists from Poland, the Netherlands and Germany, Jerzy Antychowicz, Michał Reichert, Marek Matras, Sven M. Bergmann And Olga Haenen, published a study in *Bull Vet Inst Pulawy* 49, 367-373 that reported the following (abstract from the paper):

The presence of koi carp herpesvirus (KHV) infection in Poland was confirmed in common carp reared for consumption. Virus was isolated in CCB cells using cocultivation technique. The carps were experimentally infected and virus was reisolated and identified with PCR method. Monitoring of KHV in 15 carp farms showed the presence of the virus in 4 farms. We have found that lowering of the water temperature to 11-12°C could eventually provoked the recurrence of the disease symptoms in latently infected survivor carp, and thus could help to detect KHV with PCR or co-cultivation methods. Analysis of sequencing data of 484 bp fragments of KHV DNA of 4 Polish isolates revealed the complete identity in 3 cases. One of the Polish isolate differed from the remaining 3 variants by 5 nucleotide substitutions. In order to evaluate fully the importance of small differences in DNA sequences of the KHV isolates, corresponding aminoacid analysis, and subsequent virulence studies are necessary.

Jargon or abbreviations:

CCB - carp brain cells

Cocultivation - growing CCB and cells from survivor carp in the same jar. In this case, the survivor cells were leukocytes (white blood cells).

bp - base pairs (in a DNA sequence, either TA, AT, GC or CG).

In 2005, scientists from UC Davis and UC Sacramento, Mark A. Adkison, Oren Gilad and Ronald P. Hedrick, published a study in the Japanese Society of Fish Pathology, 40 (2), 53–62, 2005. 6, that reported the following (abstract from the paper):

An enzyme linked immunosorbent assay (ELISA) detected the presence of anti-koi herpesvirus (KHV) antibodies in the serum of koi or colored carp (*Cyprinus carpio*) following either natural or experimental exposures to KHV. Concentrations of anti-KHV antibodies were detected at serum dilutions as great as 1:62,500 in a population of koi kept in virus-free water for 1 year following a naturally occurring outbreak due to KHV. At serum dilutions less than 1:2,500 cross reactions with a second herpes-like viral agent Cyprinid herpesvirus 1 (CyHV-1) was detected in serum from both experimentally and naturally KHV exposed koi. Passive immunization by administration of anti-KHV antibodies from koi recovered from previous virus infections to naive koi provided only partial and transient protection to waterborne challenges with KHV. Koi that maintained high levels of serum anti-KHV antibodies after 1 year in virus-free water are deemed as suspect carriers of the virus. The identification of suspect carriers by screening of koi and common carp populations, including potential broodstocks, with the KHV ELISA should improve the ability to control this important viral pathogen.

In 2007, scientists from Israel, Arnon Dishon, Maya Davidovich, Maya Ilouze and Moshe Kotler, published a study online ahead of print in the Journal of Virology that found the following (abstract from the paper):

Cyprinid herpes virus 3 (CyHV-3) previously designated carp interstitial nephritis and gill necrosis virus (CNGV), and koi herpes virus (KHV), is the cause of a worldwide mortal disease of koi and carp. Morphologically the virus resembles herpes viruses, yet bears a genome of 277-295 kbp, which is divergent from most of the genomic sequences available in the GenBank. The disease afflicts fish at the transient seasons, when the water temperature is 18-28°C, conditions which permit virus propagation in cultured cells. Here we report that infectious virus is preserved in cultured cells maintained for 30 days at 30°C. CyHV-3-infected vacuolated cells with deformed morphology converted to normal, and plaques disappeared following shifting up of the temperature, and reappeared after transfer to the permissive temperature. Viral propagation and viral gene transcription were turned off by shifting cells to the non-permissive temperature. Upon return of the cells to permissive temperature, transcription of viral genes was reactivated in a sequence distinguished from that occurring in naïve cells following infection. Our results show that CyHV-3 persists in cultured cells maintained at the non-permissive temperature and suggest that viruses could persist for long periods in the fish body, enabling a new burst of infection upon shifting to permissive temperature.

Abbreviations:

kbp – kilo-base pairs (thousands of base pairs)

So what does all this mean?

Antychowicz *et al.* gives us a real clue as to where the virus may be “hiding” when it’s not active in the fish. They were able to re-establish the virus invitro (literally “in glass” outside the fish, in glass containers) by putting white cells from survivors of KHV infections together in a jar where they had a growing batch of carp brain cells. This was done with white cells from the same fish where the group was unable to get the virus to grow invitro in CCB with supernatants (the liquid) from gill and kidney homogenates (think “blender”). BTW, Andy Goodwin said that channel catfish virus probably “hides” in the white cells of those fish when it’s not active. It’s also a herpesvirus.

Another thing Antychowicz *et al.* tells us is that this virus may be mutating and adapting to the colder waters found in Poland. The fact that they were able to re-establish the active disease in fish at 11 to 12°C (51.8 to 53.6°F), is disturbing to say the least. The virus we have been used to seeing goes “dormant” in fish and invitro at temps below 13°C (55.4°F).

Adkison *et al.*, showed that survivors of natural outbreaks and artificially induced disease almost all develop measurable anti-KHV antibodies. They speculated that fish with high antibody levels at one year post infection, are likely carriers.

They also found that passing antibodies from survivors to naïve fish conferred only a slight and temporary improvement in immunity to a KHV challenge. They concluded from this that other immune mechanisms (e.g., cellular involvement) were likely at work in the survivors.

Dishon *et al.* reported another disturbing phenomenon when KHV-infected cells grown invitro at “permissive temperatures”(18 to 28°C or 64.4 to 82.4°F) were raised to 30°C (86°F), the virus stopped reproducing. But when those cells were later brought back down to the permissive temperatures, the virus again started to replicate. They speculate this may also happen invivo (literally “in life” meaning, in this case, “in a live fish”) allowing the virus to persist at high temps but again start to replicate when the temperature is reduced. The potential “watch-outs” with this speculation is that, 1) there is no functioning immune system (one which might clear or significantly inhibit the virus in a live fish. See the findings of Adkison, *et al.* in the paragraph just above this one) in the lab jars and 2) temperature ranges for optimum virus growth in cell cultures tend to be 2-3°C (1.8 to 2.7°F) wider than those in fish (Gilad *et al.*, 2003). All this said, it’s yet another finding that makes us think this bug may have numerous mechanisms to survive and later come back to haunt us.