GOLDFISH HERPES UPDATE

By Dave Mandley

Since October of 2004 I have been involved with the research on the virus. My fish room has become an experimental laboratory for some of the research. Recently we did an experiment on contamination and the spread of the virus in a closed facility. The experiment went in two directions. For one I needed wild caught fish so I purchased 200lbs of Common Goldfish from a commercial fisherman in Lake Eyrie. For the other experiment I brought home 200 matt Shubunkins I knew to be virus free.

I placed 50 Shubunkins into several tanks in my fish room with fish which were known to be contaminated with the virus. After 5 days I treated the new arrivals with heat and salt, 84F and one tablespoon of salt per gallon, for 2 weeks. Thinking that the fish had been infected then cured, I moved the 50 fish to an outdoor pond. The water temperature in the pond was in the '70's and the pond also contained 6 fish which were known carriers of the virus. Our thinking was we introduced the virus indoors and then heat treated, similar to modified live parvo virus shots for dogs. Once outdoors, the fish started dying in 3 days. I sent 6 fish to the lab for testing (viral, bacterial, parasite write-ups). The answer came back that my Shubunkins had virus throughout their entire body; every organ and all tissue tested positive. This was even worse than the original outbreak showed. I retested my indoor Shubunkins, minimal contamination, not enough active to be a carrier fish. That is why the 50 Shubunkins were not infected indoors. Question - Why did my outside Shubunkins infect the new arrivals? The only difference was that my outdoor fish had body and gill flukes. I put the fish outside on 23rd June when the temperature was too warm for the virus to be active, but the flukes seemed to be the agent or catalyst to spread the virus. This may be why when treating fish, salt and high temperature become important. The salt may be killing the agent (the flukes) and the temperature may be helping the fish's immune system to fight off the virus or bacterial infection.

We know that the virus makes the fish anaemic; parasites biting into already weakened and bleeding fish may be the cause of death in some of the fish. More research needs to be done on the parasites themselves.

Back to my outdoor Shubunkins. Fish were dying rapidly but with two treatments of formalin and malachite green they stopped dying. There have not been any more losses in the pond and I have added new fish to this pond regularly every 3 weeks.

I asked Dr Goodwin for an article to be written for the Goldfish societies. He responded nicely. Dr Smartt and Dr Bonar also have written articles to help us understand this viral problem. Thanks to people like these gentlemen we can understand and not be afraid of this fish problem.

I am hearing of outbreaks in the United Kingdom with experienced goldfish breeders. Some of the fish which were infected have been imported to the United States: not a problem, I believe that we and Europe have been fighting this virus for at least 20 years. We just didn't have a name for it.

My fish room is divided into two halves – Infected and Naive. I use many different gravel cleaners to empty my tanks, keeping two or three in bleach water when not in use. I have plastic partitions between tanks to eliminate splashing and assigned certain nets to specific areas.

My earthen ponds have all been tested for virus. Interestingly, I have 11 acres free of virus and 4 acres with virus. As the level of testing increases, we may find the virus present in more ponds. I will monitor all these ponds and send you up-dates periodically.

Fish that have been tested to be couriers have been treated and then allowed to spawn. To date none of their young have tested positive to the virus meaning there is no Vertical Transmission. This is contrary to the commercial farms tested. My indoor breeding is with two or three fish whereas commercially thousands of fish participate and some breeders in the group may be active with virus causing vertical transmission.

This virus is not new and is nothing to be embarrassed about. Indeed in her writings during the '60's and '70's Daphne Morris frequently referred to the possibility of the existence of a "lethal gene" in her mock metallic Bristol Shubunkins. This could (or could this) have been the first reported case of "the virus". Nevertheless I commend the gentlemen who have come forward to say that they have a problem and have had fish tested. The more we learn about its distribution and the severity of outbreaks the quicker we can make progress to cure or arrest the virus, or possibly just learn how to live with it.

THE GOLDFISH HERPES VIRUS

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In 1995, the Japanese first reported a disease of goldfish caused by a herpes virus. They described the disease as "goldfish haematopoietic necrosis" and the virus is now known as Cyprinid Herpes virus 2 (CyHV-2). In the years since 1995, there were reports of CyHV-2 in the US, Taiwan, and Australia. Diagnosis of the disease in all of those cases was made by identifying herpes viruses using an electron microscope. This is a difficult and expensive technique rarely used by fish disease laboratories and it may be that many cases were missed. In an effort to better understand CyHV-2 and improve diagnosis, a new DNA test (PCR) for CyHV-2 has been developed in our laboratory at the University of Arkansas at Pine Bluff. This new assay enables fast, sensitive, and specific CyHV-2 diagnosis and recent studies using this assay have greatly increased our understanding of this goldfish disease.

The CyHV-2 virus attacks the tissues in goldfish that make blood cells. During active infections, the destruction of these tissues leads to severe anaemia. Afflicted fish develop pale gills, become lethargic, and die. Total losses from an outbreak may be quite high. Cases occurring in the US have

involved fish farms, small breeders, and individual pond owners. The cases have come from the east and west coasts as well as the central US. Goldfish are the only known hosts of the virus.

Studies investigating fish herpes viruses are often made difficult by the very nature of these viruses. Herpes viruses often persist at very low levels in disease survivors. These low levels can be very difficult to detect, but these carriers are very important because they might at some later time release virus and infect new fish. Difficulties in detecting carrier fish and uncertainties about disease transmission are the main reasons why progress in diagnosing and controlling Koi herpes virus (KHV) has been so slow. Fortunately, using our new assay we have been more successful in studying CyHV-2.

In our recent work, we have shown that the virus is common in healthy fish and is often detectable even in brood fish that are several years old. This implies that the virus is typical herpes and persists in healthy fish after infection. Work has not yet been done to show that these survivors infect healthy fish, but this does clearly occur with other fish herpes viruses. We do have good evidence that infected brood fish can pass CyHV-2 to their offspring, even in eggs that have been treated with disinfectants like formalin and iodine. Thus, the early evidence is that survivors are carriers and that they can pass the virus to healthy fish. These characteristics will make it a difficult virus to control or eradicate.

The most important unanswered question about CyHV-2 is whether or not it is really an important disease problem of goldfish. There are reliable reports of serious fish kills associated with the virus, but these reports are quite rare when you consider that the virus appears to be quite common worldwide. One possible explanation for the shortage of CyHV-2 disease reports is that fish kills are quite common, but that diagnosis by electron microscopy is so difficult that most cases have gone undetected. The other possibility is that the virus is widespread, but only produces serious fish losses under fairly rare host and environmental conditions. Both of these possibilities can be clearly demonstrated in other closely related fish herpes viruses.

The closely related Koi herpes virus is a nasty virus that is highly contagious and produces severe mortality when fish are exposed for the first time. If CyHV-2 is like KHV, we would expect that most introductions of the virus would be accompanied by high mortality. The other possibility is that CyHV-2 is more like the channel catfish virus (CCV). The best evidence is that CCV is extremely common in catfish populations but that it only produces disease under rare combinations of host and environmental conditions (high temperatures, handling, and fish 1-4 months old). Based on what we know of CyHV-2, I suspect that it behaves more like CCV than the contagious and nasty KHV. However, it is important to remember that both CCV and CyHV-2 can kill a lot of fish under the right conditions. Unfortunately, for the goldfish virus, those conditions are not well known.

There is still much to be learned about CyHV-2. We need to know more about the disease and its transmission, but other questions may be more

important. For example, it is clear that the virus can kill fish and will be difficult to eradicate, but we need to know if the benefits of eradication will outweigh the costs. We also need to learn more about what conditions lead to CyHV-2 fish kills and how to prevent outbreaks in infected populations. In the meantime, it is important for breeders and hobbyists to know that the disease exists, that the virus is widespread, and that the signs of the disease are anaemia and pale gills. It is too early for us to consider any attempts to eradicate the disease or to limit fish sales or movements based on the detection of the virus in healthy fish by PCR. Fish with active CyHV-2 disease may be treated with elevated temperatures (greater than 82° F), but you must keep in mind that survivors of the outbreak are probably carriers of CyHV-2 that could, under some conditions, transmit the virus to other fish.

So what should you do if you suspect CyHV-2? First, remember that there are many ways to kill goldfish and that dead goldfish, even anaemic goldfish, are not proof that CyHV-2 is the problem. Even if the virus is detected in the fish by PCR, the diagnostic data must be carefully examined to determine if the fish actually had CyHV-2 disease, or if it was instead a CyHV-2 carrier that was dying of something else. Also remember that current diagnosis is based on PCR tests. You will need to work with a veterinarian with skills in fish disease, or with another fish health professional or diagnostic laboratory, to get the PCR testing done. In an emergency, freeze a very freshly dead fish for later PCR analysis, but remember that other common goldfish diseases can probably not be detected in frozen specimens.

For more scientific information about CyHV-2, see:

Chang P-H, Lee S-H, Chiang H –C, Jong M-H (1999) Epizootic of herpes-like virus infection in goldfish, Carassius Auratus in Taiwan. Fish-Pathol 34: 209-210

Goodwin AE, Khoo L, LaPatra SE, Bonar C, Key DW, Garner M, Hanson L. (in press) Goldfish Haematopoietic Necrosis Herpes virus (Cyprinid herpes virus 2) In the USA: Molecular Confirmation of Isolates from Diseased Fish. J Aquat Anim Health

Groff JM, LaPatra SE, Munn RJ, Zinkl JG (1998) A viral epizootic in cultured populations of juvenile goldfish due to a putative herpes virus aetiology. J Vet Diag Invest 10: 375-378

Jung SJ, Miyazaki T (1995) Herpes viral haematopoietic necrosis of goldfish, Carassius Auratus (L.) J Fish Dis 18:211-220

SCIENTIFIC ADVISER'S JOTTINGS

Goldfish Herpes Virus

Joe Smartt

The recent confirmation of the presence of this virus in Britain in the wake

of its incidence in the United States clearly calls for a considered response from all those in the country concerned for the wellbeing of the goldfish. The great problem we have to face is that we really have so very little background information and most of that is anecdotal. An outbreak becomes apparent when sudden catastrophic losses occur when even complete populations can be wiped out. The characteristic symptoms developed occur in the gills which lose their normal bright red colour and death follows.

We do need a great deal more information as to how widely spread the virus is and in what circumstances disastrous outbreaks occur. In the past there have been sudden incidents of very heavy losses which have been successfully nipped in the bud by the prompt action taken. This has involved isolation of the affected ponds and tanks with thorough disinfection of facilities and effective quarantine. It is a matter of conjecture whether these incidents have been due to herpes virus attack or not but in those with which I have been acquainted no certain cause has been identified. It is a distinct possibility that the cause had been an unidentified virus. If those apparently isolated and infrequent incidents were in fact due to a virus the implications are that this virus has been in this country for a number of years and that it becomes apparent only in special circumstances which have acted as a trigger to rapid replication of the virus and development of disease. This situation would develop if the virus were carried by individuals which did not develop symptoms unless and until the trigger operated.

The modes of dispersal of the virus could include in addition to symptom less carriers water borne virus particles and the contamination of equipment by them. The most probable point of entry for the virus is the gills themselves, these appear to be the most easily penetrable surfaces for a pathogen of this type. It is also possible that the virus could be carried mechanically by parasites such as Argulus and flukes.

The information which would be initially most helpful could be produced by a sampling of goldfish populations throughout the country to determine just how widespread it is. We also need to gather reliable information which would enable us to deduce its source. The general view is that it probably came in imported fish from the Far East, which appears to be the world's prime source of novel and virulent viruses affecting all manner of vertebrates. Its occurrence in the Far East would be strong presumptive evidence that it originated there as the movement of fish is predominantly in one direction, very few fish are moved from the West to the Far East. Its current widespread distribution which has been achieved without the occurrence of trails of widespread destruction supports the notion that it has spread widely by movement of individuals which are carrying the virus without developing obvious symptoms. Had it spread recently and shown a consistent high level of virulence the expectation would be that an obvious path of movement would have been the result. The nature of its distribution and the sporadic outbreaks increases the difficulty of tracing its spread and dating its apparent origin and the times of introduction to those places where it now occurs.

The sudden onset of the disease in the outbreaks recorded so far has provided little or no prior indication of its presence. What appear to be perfectly healthy populations suddenly develop anaemic gills followed in a matter of a very few days by death. This makes the suggestion of the disease being triggered by an extraneous factor plausible. As to the nature of possible triggers we can only speculate, the most likely are environmental. Any adverse environmental shock producing loss of condition is one possibility; it is possible that the attack of another pathogen could also produce this effect. It may be that a latent infection held in check by the immune system might become apparent if this was compromised. The nature of the sudden onset of the virus attack would be consistent with its sudden rapid replication and release; this could overwhelm the host immune system. At lower virus concentrations it may be able to invade but be held in check by the immune system. This situation could continue indefinitely until such time as rapid virus replication was triggered.

The hypothesis developed here is consistent with known facts and probabilities and could provide the basis for a sensible reaction and response. The current situation is such that most people are at a loss as to how best they should react. Any response needs to be considered very carefully. Hasty and panic reactions should be avoided as these could be quite useless. When an outbreak occurs in any establishment affected fish and their immediate contacts should be isolated and the outbreak localised. All appropriate bio security measures should be adopted with the strictest possible hygiene being observed. At fish shows measures taken during the SVC scare some years ago would be entirely appropriate.

Most of the information we have on recent outbreaks has come from the United States via our American member Dave Mandley who has been supplying stock to a group of virologists and collaborating closely with them. This group has seven members one of whom, Dr Chris Bonner, is based at the Cleveland Zoo Aquarium, Ohio. They have recently published a scientific paper concerned with the identification of virus isolates by sophisticated DNA molecular techniques. These are by their very nature laboratory based investigations which will need to be complemented by studies in the field in which it is hoped that the collaboration of relevant fish farmers can be secured.

Laboratory studies have been refined to the extent that now the virus can be detected at quite low concentrations. There is a problem here in that failure to demonstrate presence of the virus in an individual is not a cast iron guarantee that it was virus free. Identification of the virus is usually carried out on fish which have died or been sacrificed and body tissues extracted and assayed for the virus. Ideally we need to be able to identify the virus from blood or small tissue samples from live fish which can survive the procedure and hopefully serve to establish virus free stocks.

The next logical stage, once a virus free stock is obtained, would be to trace the course of deliberate infections in terms of disease development and the circumstances in which the full blown diseases develops or can be induced. With the information derived from such studies it should be possible

to devise effective strategies to contain and/or control disease outbreaks.

At the present time amateur breeders and commercial producers are tending to play their cards pretty close to their chests for the very good reason that fish pathologists might advocate a strategy of virus eradication. If the hypothesis developed here is correct such a strategy would be most unlikely to succeed; the virus has been identified virtually from coast to coast if the information I have received is correct. Effectively the virus is now endemic in the USA and probably also in much if not most of Europe and Asia. What are needed most urgently are workable procedures for the control of development of the disease such as the reduction of stress in shipping to the absolute minimum and in the course of import quarantine. It is certainly in the interests of exporters, importers, breeders and keepers of goldfish to cooperate in developing an effective system of controlling a disease which we may not in the short term at least be able to eliminate.

Another question which needs to be explored is the status of individuals which have contracted and developed the disease but have recovered. In addition we do not have any information as to whether all goldfish as individuals are equally susceptible to the disease; does resistance or tolerance occur? These are important issues which need to be resolved. Others which could be usefully investigated are the longer term effects of the disease on individuals which show apparent recovery. How does it affect the longevity of fish and their reproductive capacity? How is it transmitted from parents to offspring? Is transmission vertical via egg and/or sperm or is it essentially passive in the water? It is possible that the easiest way to establish virus-free stocks could be to strip disinfected brood stock out of direct contact with water. Of course once a virus-free stock is established the problem may be to keep it so. Whether eventually it will be possible to certify consignments of fish as virus free is a moot point; one certainly hopes that it is not necessary to do so from a practical and commercial point of view.

The identification of this virus as the cause of sudden, catastrophic loss of fish stocks may well have resolved many past disasters which have overtaken breeders. It also has provided a new if rather complex field of investigation for fish virologists and pathologists. We can only hope that they receive adequate support for the necessary research and investigation.