INTERNATIONAL WORKSHOP ON KOI HERPESVIRUS

London
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INTERNATIONAL WORKSHOP ON KOI HERPESVIRUS

Executive Summary

1. **The Koi Herpesvirus International (KHV) Workshop was held on the 12th and 13th February 2004 in London, UK.**

2. **The workshop was attended by 45 stakeholders from 8 countries representing industry, science, regulators and policy makers.**

3. The aim of the workshop was: To raise the general awareness amongst regulators, scientists and the industry of the current status of Koi Herpesvirus with respect to its management, identification and potential treatment.

4. The objectives of the workshop were to:
   - Assess the current status of the disease in terms of its impact on the ornamental fish sector
   - Discuss the practical management of the disease throughout the ornamental fish supply chain
   - Explore the various strands of research and development into KHV and assess the potential for co-ordinated international research effort
   - Seek to make recommendations for the future management of KHV and research requirements

5. Delegates took part in one of three discussion groups each of which addressed the following questions:

   Group 1
   - What are the tools required and criteria needed to substantiate KHV freedom at a site level to enable safe trade to continue? How can these approaches be used to contribute to OATA current best practice? (Chaired by: ***Keith Davenport***)

   Group 2
   - Standardization of detection procedures - can we agree on what are the best methods and how they can be applied? What new approaches are needed? (Chaired by: ***Ron Hedrick***)

   Group 3
   - Identifying key information gaps. What research is underway and what is needed? Can efforts be coordinated and what funding sources are available? (Chaired by: ***Barry Hill***)

6. **Group 1:** Concluded that the industry should develop a self-audit process as soon as possible, which should include recommendations for diagnostic tools, development of a Code of Practice, promotion of the self-audit process, a list of self-audit compliant sites and list of compliant importers. The Ornamental and Aquatic Trade Association together with the Professional Koi Dealers Association agreed to take forward these initiatives.

7. **Group 2:** Highlighted the information gaps that exist with a range of screening techniques. The Group suggested a detailed regime for screening for KHV, including suggested techniques, minimum age of fish, multiple year class tests, sample sizes and tissues to be sampled. The Group discussed standardisation of methods and agreed that a ring trial of current PCR methods is required. A future requirement will be the publication of PCR based on sequence from known genes. Techniques for presumptive and confirmatory test for the clinical disease were discussed and it was agreed that all methods and reagents need to be standardised and proficiency testing performed as part of a ring trial.

8. **Group 3:** Highlighted the need to establish whether wild carp populations are at risk and if so, under what conditions. In addition, there is a need to establish whether this virus is

endemic within the EU. The risk posed by fish surviving KHV needs to be assessed. Suitable detection methods are required to demonstrate absence of the virus. More information on the serology of KHV is required, together with a better understanding of the fishes immune response to the virus. Vaccine development was considered a priority. The Group attempted to collate a list of related R&D projects from workshop participants, and potential avenues of funding and future collaborative links were discussed.

9. General discussions confirmed the need for international co-operation to combat this disease. The setting up of a "steering group" to facilitate co-operation and co-ordination of activities was suggested and staff from CEFAS Weymouth took a lead in initiating this process. It is likely that resources will be required to sustain the activities of this group.

10. OATA committed to adding the names of all the workshop delegates to it's KHV Internet List Server, to facilitate comment and exchange of information.

11. It was suggested that a bid for EU funds would be an appropriate way forward to help support the range of R&D activities required to understand, contain and hopefully eradicate KHV.

12. An electronic version of this report, together with associated PowerPoint presentations is available on the following websites:
http://www.defra.gov.uk/science/Publications/Default.asp
http://www.frmltd.com/resources.htm

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INTRODUCTION

Aim: To raise the general awareness amongst regulators, scientists and the industry of the current status of Koi Herpesvirus with respect to its management, identification and potential treatment.

Objectives:

- Assess the current status of the disease in terms of its impact on the ornamental fish sector
- Discuss the practical management of the disease throughout the ornamental fish supply chain
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- Seek to make recommendations for the future management of KHV and research requirements

Background

Koi Herpes Virus is having a significant impact on fish welfare and the conduct of the international trade in ornamental fish. The workshop was developed in recognition of the need to bring together key international stakeholders including members of the industry, scientists and regulators to ensure a common understanding of the status of this disease, its management and potential treatment. The KHV International workshop provided a focus for disseminating the latest information on the impact of the disease with respect to the ornamental industry and acted as a forum for discussing the latest practical management measures adopted to restrict the spread of the disease. Those actively involved in research into diagnostics and treatments for KHV were encouraged to exchange information, co-ordinate their activities and explore the possibilities for international collaboration. The workshop helped to identify key research requirements in this field.

Day 1 - Presentations

Initial Isolation and Characterization of a Herpes-like Virus (KHV) from Koi

Hedrick, R.P., Gilad, O., Yun, S.C., McDowell, T.S., Waltzek, T.B., Kelley, G.O., Adkison, M.A.
Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, California 95616 USA

In September of 1998 our laboratory was asked to investigate epidemics characterized by high mortality occurring in koi populations in both the USA and Israel. Either live fish or frozen tissues arrived from both locations in September and November of 1998. In addition, tissues fixed for both light and electron microscopy from fish in Israel and the USA were obtained for examination. The virus observed and then isolated in 1998 from these initial samples has become the focus of a worldwide concern for the health and welfare of both captive and wild populations of koi and common carp (Hedrick et al. 2000).

The development of the koi fin (KF-1) cell in our laboratory in 1997 made possible the isolation of KHV as commonly used cell lines by fish virology laboratories were not susceptible to the new virus. The virus was isolated from numerous tissues of fish with signs of the disease, including the gill, kidney, spleen, gut and liver. The virus induced cell fusion and intense cytoplasmic vacuolation in KF-1 cells within 5 days after inoculation at 20^{o}C. More complete cytopathic effects (CPE) were evident at 7 – 10 days and progressed to involve all the cells after 14 days. The virus grew in the KF-1 cell line at temperatures from 15 – 25^{o}C with an optimum at 20^{o}C (Gilad et al. 2003). Electron microscopy of the virus present in and released from infected KF-1 cells was shown to possess a morphology and size consistent with viruses in the family *Herpesviridae*. The virions were composed of an inner capsid with icosadeltahedron symmetry of approximately 100 - 110 nm in diameter. Mature virions contained a loosely applied envelope giving the virion an overall diameter of 170 – 230 nm. Identical virus particles were observed in tissues from koi from fish involved in the epidemics in Israel and the USA. Based on the morphology and size of the virus and the sequential development in the host cell nucleus, the new virus was initially designated KHV for koi herpesvirus (Hedrick et al. 2000). The same agent was isolated subsequently from koi and common carp by Ronen et al. (2003) although they preferred the designation of carp nephritis and gill necrosis virus (CNGV) for the agent.

That KHV was the cause of the disease epidemics was established by experimental demonstrations that the virus grown in KF-1 cells could reproduce the disease among koi exposed by either bath or intraperitoneal injections in the laboratory (Hedrick et al. 2000). That the virus differed from *cyprinid herpesvirus* 1 (CyHV-1) or the agent known to cause carp pox (Sano et al. 1995) was suggested by differences in the diseases caused by both agents and when anti-CyHV-1 antibodies failed to react with KHV in immunofluorescence assays. Differences in the virion proteins and genomic sequences was additional proof the two viruses were different (Gilad et al. 2002).

The large size of the genome of KHV or CNGV, which is estimated at 277 kbp, exceeds that of 250 kbp known for members of the family *Herpesviridae* (Ronen et al. 2003). While genome size is not currently considered a criterion for placement or exclusion of viruses in the family, the differences in sequences so far obtained for KHV have shown little similarities to those known from herpesviruses from birds or mammals. This has led to a reasonable debate on taxonomy of KHV that remains unresolved. Certain proposals however, are developing that may involve considerable taxonomic changes in the family *Herpesviridae* which in turn may allow more specific assignments to viruses such as KHV.

Since the initial isolation of KHV in 1998, a number of new and improved methods have been developed to detect the virus. That isolation of the virus was difficult was shown early and thus other detection methods have been rigorously pursued. The polymerase chain reaction (PCR) method has proven to be an effective means to detect viral DNA in a number of fish tissues from animals during the acute disease and following recovery. As many as 7 different PCR tests for

KHV are currently in use in different laboratories throughout the world (Gilad et al. 2002, Gray et al. 2002, H. Bercovier, K. Way, M. El-Matbouli pers comm.). These tests have greatly improved the ability to detect evidence for the virus but leave unresolved whether carriers with persistent or latent infections occur following acute disease. The detection of anti-KHV antibodies in the serum of koi and common carp is currently being used as both a diagnostic and research tool to demonstrate prior exposure of fish to the virus.

Despite major progress in our understanding of KHV, many important questions remain to be answered. Among some of the more important questions are:
1) Where did this virus come from (from an evolutionary and geographic standpoint)?
2) What are the potential reservoirs and by which means has the virus spread?
3) Is the virus able to establish latent or persistent infections?
4) What mechanisms underlie the lethal nature of the virus?
5) Can we develop safe and effective methods to prevent the disease?

Answers to these and many more questions will come from in depth research studies of the virus and disease in the field and laboratory. The development of guidelines and potentially regulations that effectively control pathogens in ornamental fish must also be considered. Discussions between scientists, resource managers, ornamental trade representatives and concerned hobbyists are the first important steps in controlling this viral disease and logically will be the most important outcome of the international conference.

Gilad O, Yun S, Andree KB, Adkison MA, Zlotkin A, Bercovier H, Eldar A, Hedrick RP (2002) Initial characteristics of koi herpesvirus and development of a polymerase chain reaction assay to detect the virus in koi, *Cyprinus carpio koi*. Dis Aquat Org 48:101-108.

Gilad O, Yun S, Andree KB, Adkison MA, Way K, Willits NH, Bercovier H, Hedrick RP (2003) Molecular comparison of isolates of an emerging fish pathogen, the koi herpesvirus, and the effect of water temperature on mortality of experimentally infected koi. J Gen Virol 84: 1-8.

Gray WL, Mullis L, LaPatra SE, Groff JM, Goodwin A (2002) Detection of koi herpesvirus DNA in tissues of infected fish. J Fish Dis 25:171-178.

Hedrick RP, Gilad O, Yun S, Spangenberg JV, Marty GD, Nordhausen RW, Kebus MJ, Bercovier H, and Eldar A (2000) A Herpesvirus associate with mass mortality of juvenile and adult koi, a strain of a common carp. J Aquat Anim Health 12:44-57.

Ronen A, Perelberg A, Abramowitz J, Hutoran M, Tinman S, Bejerano I, Steinitz M, Kotler M (2003) Efficient vaccine against the virus causing a lethal disease in cultured *Cyprinus carpio*. Vaccine 21:4677-4684.

Sano T, Fukuda H, Furukawa M, Hosoya H, Moriya Y (1985) A herpesvirus isolated from carp papilloma in Japan. Fish Shell Pathol 32:307–311.

Ron Hedrick, Presentation 1 – Rapporteur's Notes

Barry Hill. **Q:** What was the time interval between injection of immune fish serum and challenge? **A:** 24 hours. Serum was undiluted; 0.1 ml was injected intraperitoneally per fish. With channel catfish virus the same procedure gave better protection. Only partial protection to KHV disease afforded by passive immunisation with serum antibodies. This indicates that the cellular immune response also important.

Prevention of a mortal disease of carps induced by the carp interstitial nephritis and gill necrosis virus (CNGV)

Moshe Kotler, Ariel Ronen, Berta Levavi-Sivan, Marina Hutoran, Yechiam Shapira, Michael Steinitz, Ayana Perelberg, and Eli Pikarsky

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Summary:
Massive mortality of Koi and Common carp - *Cyprinus carpio* species - was observed in many farms throughout Israel, resulting in severe financial losses. This lethal disease is highly contagious and extremely virulent, but morbidity and mortality are restricted to Koi and Common carp populations. We isolated a carp nephritis and Gill necrosis virus (CNGV), which is the etiologic agent of this disease. The virus propagates and induces severe cytopathic effects five days post infection in fresh Koi fin cell cultures (KFC). The virus harvested from KFC cultures induced the same disease with mortality of 75-95% upon inoculation of naive Koi and Common carp.

Electron microscopy revealed viral cores with icosahedron morphology of 100-110 nm resembling the herpes virus. Electron micrographs of purified pelleted CNGV sections, together with sensitivity to ether and Triton x 100 suggest that it is an enveloped virus. However, the genome of the isolated virus is a double-stranded DNA molecule of 250-300 Kbp, larger than that of known *Herpesviridae* members. The viral DNA seems highly divergent and bears only small fragments (16-45 bp) similar to the genomes of several DNA viruses. We suggest, therefore, that the etiologic agent of this disease may represent as yet unclassified virus species endemic to carpinoids.

Carps, exposed to the virus at 23 ^{0}C for 3-5 days and then transferred to the non-permissive temperature of 30 ^{0}C, became resistant to a challenged infection and their sera demonstrated a high level of virus specific antibodies. We have isolated attenuated non-pathogenic viruses that render virus-vaccinated carps resistant to the disease. Furthermore, vaccinated fish developed high levels of antibodies against the virus. We suggest, therefore, that this attenuated virus could be used as a live vaccine for the eradication of the mortal disease afflicting common and ornamental carp fisheries in many countries.

We examined the pathobiology of this disease in carp using immunohistochemistry. We found large amounts of the virus in the kidneys of sick fish, and lesser amounts in liver and brain. A rapid increase in the viral load in the kidneys was detected using both immuno-fluorescence and semi-quantitative PCR. Histological analyses of fish at various times after infection revealed signs of interstitial nephritis as early as 2 days post-infection, which increased in severity up to 10 days post-infection. There was severe gill disease evidenced by loss of villi with accompanying inflammation. Minimal focal inflammation was noted in livers and brains.

Two diagnostic methods for identifying the CNGV in alive fish are in use in our laboratory: Using PCR with authentic primers is applied on blood, kidney or gills DNA samples and immunological diagnostic kit. The immunological kit is design to be a simple, rapid and low cost diagnostic kit, which will be appropriate for retailers as well as hobbyists to identify the CNGV in presymptomatic fish. We believe that these means will be instrumental in preventing the distribution of sick fish world wide.

Moshe Kotler, Presentation 2 – Rapporteur's Notes

Keith Davenport. **Q:** Are the Israeli and US viruses the same?
A: Yes.

Ellen Ariel. **Q:** The attenuated virus was not pathogenic, but it is antigenic. Have any molecular comparisons been done?
A: Yes and no differences were seen. The problem is that there may be point mutations, but they may not be identified because the full sequence is not known. An attenuated DNA virus has a 10^{-4} to 10^{-5} chance of reversion, therefore the chances of it happening are small. Even if it does happen in one fish, the other fish around it will have been immunised. Also mentioned that the virus is irradiated – then used polio as an example of a very safe vaccine.

Chris Seagrave **Q:** Are you saying that your vaccine is a magic bullet ? If it is so simple why not just drop attenuated KHV into the water and obtain 100% protection for all the carp in the pond.

You say this vaccine gives 100% protection, but that does not occur with other fish viruses. Why not?
A: Not fully recorded – retrospective answer given as follows:
To achieve immunization you need to bath the fish for at least 45 min. in water containing about 10 PFU/ml. To do such a procedure in large volume of water is not economic. Moreover, we already know that the attenuated virus is poorly transmitted from fish to fish (actually 4-5 days post immunization no virus is released from the infected fish). I [Moshe Kotler] do not remember if I said 100%. If so I would like to correct myself: Our vaccine preparation does not kill any of the immunized carp and 90-100% of the fish survived the challenge infection. The Koi are more sensitive: 85%-90% survived the immunization and about 95 of those were resistant to the challenge infection.

Koi herpesvirus : Diagnostics and research at CEFAS Weymouth laboratory 2000 – 2003.

Keith Way, Rose-Marie LeDeuff, David M Stone, Kevin L Denham and Sophie St-Hilaire.

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Koi herpesvirus (KHV) was first isolated in the UK in the year 2000 during a disease outbreak at an ornamental fish import and retail site in northern England. Outbreaks of clinical disease with signs similar to KHV disease had been reported in 1998 and 1999 but techniques to confirm diagnosis were not available to the CEFAS laboratory until 2000. Further KHV outbreaks were confirmed in each of the next 3 years using PCR- and cell culture-based assays developed by the School of Veterinary Medicine, UCAL, Davis, USA. Furthermore, and most significantly, during 2003 KHV was isolated for the first time from wild carp in fisheries in England.

Research on KHV at CEFAS Weymouth has largely focussed on virus characterisation studies, and the development of diagnostic techniques and research tools to assist disease pathogenesis and epidemiological studies.

Approximately 50kbp of the KHV genome (30% of the total genome based on a genome size of 150kbp) has been sequenced and analysed. A large number of putative genes have been identified, many of which, share significant homology with genes found in channel catfish virus (CCV) and other herpesviruses. The most relevant are two neighbouring KHV genes in a 7.4 kbp fragment that share significant homology with the CCV helicase (ORF 25) and capsid proteins (ORF 27). The evolutionary relationship for the other genes in this region remains speculative, but since the overall gene arrangement for this region is also similar to the arrangement seen in the CCV genome, it strongly indicates that KHV and CCV are related herpesviruses.

Research on techniques to improve diagnosis of KHV have included validation of the KHV PCR protocol (Gilad et al. 2002) as a routine diagnostic tool and development of more sensitive PCR assays to enable detection of virus DNA in sub-clinical carrier fish. The research tools that have been developed include an *in situ* hybridisation (ISH) assay for localisation of KHV DNA in infected tissues and a modified PCR assay for detection of DNA in archive fixed and paraffin-embedded tissue (archive-PCR). The archive-PCR has been successful in detecting and confirming KHV DNA in paraffin-embedded koi carp tissues sampled during disease outbreaks in 1999 and in tissues sampled from carp mortalities in earlier years. The implications of these findings will be discussed.

Areas identified for future research at CEFAS include the validation and use of serological assays to identify carp populations that have been exposed to KHV. This will assist in determining the prevalence of virus carriers in a population and in establishing the range of water temperatures, at which KHV antibodies are detectable. It is planned that these studies will include non-lethal sampling methods and incorporate techniques for detecting virus or virus DNA in samples

including blood and tissue biopsies. Details of all research developments and their application and future research areas will be discussed.

Keith Way, Presentation 3 – Rapporteur's Notes

Andy Goodwin. **Q:** Did you check the experimentally infected goldfish by PCR post challenge?
A: No. We haven't looked for carrier fish but a study is planned to investigate this.

Ron Hedrick. **Q:** Does the type of fixative affect the ISH or archive PCR ?
A: All of the development work has been done on NBF-fixed tissue. In the future it is planned to also take tissue samples into 70% alcohol to better preserve the DNA.

Marcus Biffar. **Q:** Could the nested PCR be used to detect carriers?
A: No. This is a recent finding [nested PCR-positive common carp]. It is difficult to validate this type of work. Looking at ISH or alternative technique to provide a way of validating the method.

KHV and possible existence of a carrier state

Herve Bercovier, Avi Eldar and Amir Alotkin

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Infection with KHV is considered the most important risk factor for the Israeli carp and koi industry, with losses that head over 50% of the total production. To improve our understanding of the biology of the virus, by exploring the likelihood of a carrier state, we carried out a double blind survey. The presence of KHV of KHV DNA in a variety of fish submitted to our laboratory for routine tests was explored through conventional virological procedures, PCR assays and Real-Time PCR. According to the primers used, the PCR test showed different sensitivity. As expected, sensitivity varies considerably according to the methodology employed. Results pointed out a straight correlation between virus isolation and Real-Time PCR. We were able to isolate virus from KHV survivors for up to one year after infection. It is unclear if the viruses isolated from KHV survivors are related to a carrier state or to re-exposure to wild-type viruses in infected environment.

Avi Eldar, Presentation 4 – Rapporteur's Notes

Rudolf Hoffmann. **Q:** Which tissues do you use for virus isolation?
A: Kidney.
Rudolf Hoffmann. We have been able to detect carriers up to two years post infection, by virus transmission and by detecting virus antigen in the brain.

Ron Hedrick. We used the Taqman PCR and detected virus in the brain, and we could also isolate virus from the brain, but not after two-years. We have not looked at long-term virus carrier state in brain tissue. The intestine may be another tissue to use for virus detection.

Avi Eldar. The virus may be in the pond material, and there may be transmission from the water.

Oran Gilad. The viral DNA may possibly remain in a latent state in the brain.

Trevor Hastings. **Q:** What were the numbers of fish treated for the carrier work, and what was the starting prevalence? How can you say the carrier state is self-limiting?
A: These are preliminary data. There were four groups of five fish. For the real time PCR there were four fish/group.

Moshe Kotler. **Q:** What do you learn if the PCR is detecting fragmented DNA and not infectious DNA?
A: That is a question for further discussion and further work.

Edward Branson. **Q:** Have you tried stress testing for carrier fish?
A: An Israeli group did this work (Tinman et.al.) but it has never been presented. The claim is that stress does not provoke virus shedding or clinical disease.

Mordi Haimi. Cortisol was used in the immunosuppression tests and no virus was induced.

Detection and isolation of KHV in Continental Europe

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In 1997 first cases of an apparently new disease were observed in Koi, which was proven to be highly infectious. Main symptoms were swollen gills and high breathing frequency, partially local skin lesions. Histologically, massive proliferation of gill epithelia showing degeneration and necrosis and single intranuclear inclusion bodies in degenerating cells were the most prominent findings. These inclusion bodies and the electrone microscopic evidence of herpes like virus particles supported the idea of virus induced process. This was proven by the isolation of the new koi herpes virus by Ron Hedrick's group. Additionally to kois, common carp in single farms developed identical symptoms and mass mortality in Germany.

Since 2001, an adapted PCR method (originally published by Hedrick) has been routinely used. So we could examine koi and common carp from 657 different cases with or without history of suspicious symptoms. 187 of them could be proven to be infected using PCR and histology. These outbreaks were documented in fish from Germany, the Netherlands, Austria and South Africa, however some of them were reported to be fresh imports from other countries esp. East Asia and Israel. The number of positive cases increased continuously every year indicating a wider distribution and/or a higher sensibility of koi owners.

According to our experience, not only koi is endangered, but also carp farming and fishing esp. in Middle and Eastern Europe.

Rudolf Hoffmann, Presentation 5 – Rapporteur's Notes

Ron Hedrick. **Q:** Is there any evidence for the spread of KHV from farmed to wild carp?
A: We have no evidence for this, but there is evidence of spread to common carp from koi introduced to the farms.

Avi Eldar. **Q:** What is the annual consumption of carp in Germany?
A: About 20,000 tonnes from home production and the same from imported fish.

Neil Hardy. **Q:** Have you tested samples from eastern Europe for KHV?
A: No, but I will be in Budapest soon, and will discuss obtaining samples.

Neil Hardy. **Q:** Are there any restrictions on the movement of carp from eastern to western Europe?
A: No. There is free movement of carp into Germany.

Ellen Ariel. There has been no confirmation of KHV in eastern Europe, but there is no requirement to notify anyone.

Rudolf Hoffmann. Before the re-unification of Germany there had been no reports of viral haemorrhagic septicaemia virus or infectious haematopoietic necrosis virus in East Germany, but after reunification we found both viruses widespread in East Germany.

Outbreak of disease causing mass mortality in koi and common carp (*Cyprinus carpio*) in Indonesia[1]

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ABSTRACT: Since March 2002, Indonesian carp culture is facing a serious epizootic, which has caused severe economic loss and significant social impact. As of December 2003, losses were estimated at U$15 million, with total fish mortality of up to 80-95%. The disease outbreaks affected koi and common carp (C*yprinus carpio*) populations, all ages and sizes, cultured in different grow-out facilities (ponds, lakes, cages, etc.) regardless of stocking density. Epidemiological observations indicated that the first outbreak occurred from an imported koi carp that has just been transported from Surabaya to Blitar, both in East Java. The heavy rain prior to the initial outbreak made us to consider the possibility of an environmental triggering factor, such as temperature. Based on the history, clinical signs, epidemiological features, histopathology, results from the preliminary co-habitation trials and the polymerase chain reaction (PCR) detection of naturally and experimentally diseased fish, it is strongly suspected that Koi Herpes Virus (KHV) may be involved in this serious outbreak. To prevent the spread of the disease to other islands, the government of Indonesia declared Java and Bali Islands as isolated areas infected with the disease; and movement of koi and common carps from Java Island to other islands has been restricted for the quarantined animals. The Government has also taken measures to reduce the possible re-introduction of the disease through importation of koi and common carps to the country. This presentation include vital information collected in July 2002 during the initial investigation conducted by the Emergency Disease Control Task Force on a Serious Disease of Koi and Common Carps in Indonesia organized by NACA and partners, results of the on-going work undertaken by the Local Disease Task Force, and FAO's follow-up assistance through a Technical Cooperation Project.

Key words: Koi, Common carp, Koi herpesvirus, Outbreak, Indonesia

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[2] Directorate of Fish Health and Environment, Directorate General of Aquaculture, Indonesia
[3] Ausvet, Australia
[4] Network on Aquaculture Centres in Asia and Pacific, Thailand
[5] Food and Agriculture Organization, Rome, Italy

Agus Sunarto, Presentation 6 – Rapporteur's Notes

Keith Davenport. **Q:** Was the disease in Indonesia KHV or not?
A: *Wry smile but no answer.*

Keith Way. Three of the criteria for the disease being caused by KHV were met (Gross signs, Histopathology and Detection by PCR) .

Barry Hill. **Q:** Would *in situ* hybridisation show whether the disease was present? The CEFAS laboratory could do that.

Ellen Ariel. **Q:** What was done to isolate the virus?
A:. We did not have the facilities to isolate virus and so we sent samples to other laboratories. There may have been problems with the samples themselves.

Ron Hedrick. The UCAL laboratory was one that received a sample. It had spent too long in transportation, and was unsuitable for virus isolation.

Trevor Hastings. **Q:** One of your control measures was to not use infected broodstock. Is there any evidence of vertical transmission?

A: We do not have any evidence.

Q: What was the water temperature at the time of the outbreaks ?
A: It was 28°C. In the rainy season the temperature is 20°C.

Adrian Barnes. **Q:** Can you explain more about the use of antibiotics?
A: We use potassium permanganate to treat the fish and also give antibiotics.

Neil Hardy. **Q:** Do you know where in China the fish originated?
A: No. We only have information from the quarantine record that they passed through Hong Kong.

Marc Engelsma. **Q:** Was there one source of disease or several?
A: Not sure – possibly only one ?

Oran Gilad. **Q:** Was the PCR done in Indonesia?
A: Yes – but we have had problems.

Oran Gilad. You have used too much DNA. You will get better results by using less.
A: Yes, we are now doing this.

Barry Hill. Although there is no requirement to notify anyone about KHV, the Indonesian authorities notified the OIE on the suspicion that they might have KHV, and they are to be applauded for that.

Keith Davenport. Countries that have KHV must come forward.

Ellen Ariel. If this were not KHV, what difference would it make?

Global distribution of KHV with particular reference to Europe

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The KHV problems are global, especially now that Japan is also positive for KHV since May 2003. In this paper we present the current global situation, as recorded via e-mails, our network and the literature. Also the impact of KHV on a global scale will be discussed.

EUROPE:

- Belgium: The disease is present since 1999 in koi, with mortalities up to 90%. Sometimes carps showed symptoms.
- Denmark: Since July 2002 KHV positive: in carps <10 cm from a wholesaler pond KHV diagnosed by PCR on gill tissue (also SVCV was isolated). Summer 2003: two importers with Japanese koi (13-15 cm) were KHV positive from the same source. KHV diagnosis was done by PCR on gill tissue.
- England: 36 outbreaks in 2002 in a wide range of carp sizes, All detected by PCR, 9 also by virus isolation at KF-1 cells; outbreaks continued in 2003.
- Finland: no outbreaks so far, virus never isolated from koi.
- France: KHV outbreak in 2003 in carps/koi (?) from Israel (Munich KHV meeting, 2 Dec 2003, organized by R. Hoffmann). Virus isolation and PCR are both available for diagnosis.
- Germany:
    - Riems: 21 KHV cases in 2002 by PCR, virus isolation, immunofluorescence, E.M., June 2003 67 cases, since June 123 cases (4-6 cases in carp) in all sizes of koi/carp, but mostly in larger fish; mortalities 50-100% (koi) and 80-100% (carps);

2003 KHV in imported 2-3 years old carps from a non-EU European country to carp farms. Mortalities in affected farms 30-100%. Diagnosis by PCR, but no culturable KHV at KF-1 nor CCB cells. Asymptomatic fish were KHV negative by PCR.
    - Munich: regulary outbreaks: up to 60 in 2002 in all sizes of koi, partly in common carp; mortalities up to 100%. In so called "survivors" or "immunized "carps only a few deaths in combination with transport stress; method: PCR and histology.
- Austria: first outbreak summer 2003 in koi in a private pond, tested by PCR (Munich)
- Switzerland: KHV outbreak in 2003 (Munich meeting, Dec 2003).
- Poland: exported common carps were KHV positive (Riems).
- Hungary: no outbreaks sofar, and no suspicions.
- Luxemburg: KHV positive (Munich meeting, Dec 2003).
- Italy: 1 serious suspicion in juvenile koi by E.M. and histology, no CPE after freezing at –80˚C; Italy is KHV positive (Munich meeting, Dec 2003).
- Spain: no diagnosis yet and no suspicions of KHV.
- Scotland: no outbreaks so far.
- Ireland: no outbreaks so far.
- Sweden: no outbreaks so far, prevention by quarantine with virus isolation at KF-1 cells.
- The Netherlands: 2002: 6 outbreaks, by PCR, histology, of which only 1 in by virus isolation; 2003: 27 positives (koi) out of 68 samples (61 koi and 5 common carp) as detected by KHV PCR. Additionally, histopathology of gills and internal organs was done: in case of clinical outbreaks, some positive PCR results were confirmed. So far it occurred only in koi, and not in carp, mostly in larger fish. Generally the water temperature was between 20 and 27°C. At 30°C mortality was halted. KHV is considered to be endemic in The Netherlands, in import sites, pet shops, garden centres and private ponds.
- Russia: no suspicions, no outbreaks, but also no diagnosis.

ASIA:

- Indonesia since April 2002 (NACA)(confirmed by PCR : Java since May 2002, 30% mortality in carp; Sumatra since Nov 2002, 80% mortality in carp), outbreaks continued.
- Thailand: no KHV yet? It actively surveillances for KHV.
- Singapore: no KHV yet? It actively surveillances for KHV.
- Taiwan (January 2003: many outbreaks, >80% mortality in koi, no confirmation yet?)
- Philippines: KHV positive?
- Malaysia: KHV positive?
- Japan (pers.comm. M. Sano): KHV PCR tests are done since 2001. In May-June 2003 the first outbreak occurred in common carp and koi of 1-3 kg in a river in Okayama Prefecture, Western Japan. Oct 2003 acute mortalities occurred in Ibaraki Prefecture, Lake Kasumigaura (660 tons) en Lake Kitaura (200 tons), Eastern Japan. The water temp was 17°C. Phytoplankton bloom may have been a stress factor. In the mean time 22 metric tons were distributed from Lake Kasumigaura to 21 prefectures (Fukuda, pers.comm.). Mid Nov already 4 rivers were infected (Yoshimizu, pers.comm.). By end Nov 2003 mortalities reached 1200 tons in total. Clinical signs included severe gill necrosis and sunken eyes. There was new legislation in Japan since July 2003 (requirement of an infection free certificate). Ibaraki Prefecture has officially prohibited movements of common carp from the affected areas to other areas. All koi shows were cancelled for Nov 2003. By January 2004 23 of the 47 Prefectures of Japan were infected with KHV (Asahi Shimbun, 2004) and killed thousands of tons of fish. It threatens the USD 75 million ornamental carp industry of Japan further (Australian, 2003).
- China: handpicked koi from China were KHV positive (CEFAS). In Hong Kong, a KHV outbreak in 2001 killed many koi's in 2 weeks (pers.comm. G.Chu).
- Other NACA (Network of Aquaculture Centres in Asia-Pacific)-countries: Bangladesh, Cambodia, Hong Kong SAR, India, Korea (DPR), Myanmar, Nepal, Pakistan, Sri Lanka, Thailand, Vietnam. Other participating (non-member) governments include Iran, Rep. of Korea, Lao PDR: no outbreaks yet?

MIDDLE EAST:

- Israel: May 1998 KHV was first diagnosed (Tinman & Bejerano, 1999) after imports of koi from Europe. Since than it spread further. Israel uses active immunisation schemes and developed an attenuated vaccine.

AFRICA:

- South Africa: There was an outbreak autumn 2003, in which KHV was proven (Munich meeting, Dec 2003)(details not known).

UNITED STATES:

The disease is present since 1990; KHV was isolated from diseased adult koi, showing irregularly coloured gills (Hedrick et al., 2000).

SOUTH AMERICA:

- Chile: no outbreaks of KHV so far.
- No further data available.

AUSTRALIA:

No outbreaks so far.

Furthermore no data on KHV in other countries were available to the authors.

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Mark Engelsma, Presentation 7 – Rapporteur's Notes

Ron Hedrick. **Q:** Can you still grow the virus you isolated on EPC cells?
A: No. It was frozen at -80°C and we cannot recover it.

Avi Eldar. **Q:** What was the passage number of the EPC cells?
A: Approximately 70.

Keith Way. The cytopathic effect you saw on EPC cells was probably a paramyxo-like virus. It has been isolated in several laboratories, including CEFAS, the laboratory of Manfred Neukirch in Germany and in Belgium.

Sven Bergmann. **Q:** Were the cases of KHV in Denmark in koi or wild carp?
A: Koi carp.

Ellen Ariel. Spring viraemia of carp virus was also isolated from one case.

Regional Updates

JAPAN

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Koi herpesvirus (KHV) infection was registered in June 2003 as a threatening exotic disease by the Japanese law, which is intended to protect against diseases originating in other countries. The mortality rate among food common carp reared in net pens in Lake Kasumigaura, the

second largest lake in Japan, which is located in the Ibaraki Prefecture, had increased since the first of October 2003. KHV was detected in the affected fish by the National Research Institute of Aquaculture (NRIA) in late October using PCR methods of Gilad et al. (2002) and Gray et al. (2002). The Ministry of Agriculture, Forestry and Fisheries confirmed the occurrence of the massive death of the cultured common carp due to the KHV infection and properly announced the first occurrence of the disease in Japan. Following the law, the Ibaraki Prefectural governor prohibited any shipment or removal of cultured carp from the lake and ultimately ordered that all carp cultured in the lake would be destroyed by the end of March 2004.

In late October, the water temperature of Lake Kasumigaura was approximately 16 degrees C, and the losses were severe, particularly in large-sized carp. The remarkable changes in the affected fish were discoloration of the gills and clubbing of the gill filaments. Approximately 1,200 tons of common carp cultured in the lake had been lost by mid-November. The volume accounts for one fourth of the annual production from the lake, which is the largest single production area in Japan, producing more than one half of the total annual aquaculture production of food carp in Japan. Before the restrictions were ordered by the Ibaraki Prefectural governor, infected carp had been transferred to aquaculture farms, wholesalers, restaurants, and game fishing facilities. The further infections from these facilities to the carp in other facilities or river occurred in some districts through the drain of the facilities or by reselling the infected carp.

Independent of the outbreak at Lake Kasumigaura, a massive carp loss of approximately 10,000 individuals was assessed to result from the columnaris disease in rivers and a lake in the Okayama Prefecture from May to July 2003. The NRIA, however, detected KHV DNA by PCR in samples of diseased fish stored in a freezer in November. This demonstrated that KHV had been present in Japan before the outbreak at Lake Kasumigaura.

By the end of 2003, KHV-infected common and coloured carp had been reported in 23 prefectures. No occurrence of the disease has been reported since January 2004, when the water temperature dropped.

Rapporteur's Notes

Motohiko Sano, Japan

Marcus Biffar. **Q:** What is the water temperature in November?
A: 15-16°C.

Andy Goodwin. **Q:** What is the water source for the fish farms.
A: Underground water source for the koi farms.

North America - US

Otis Miller – National Aquaculture Co-ordinator USDA, APHIS – USA
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Survey of Koi Herpesvirus (KHV) in the United States

According to the Census of Aquaculture conducted by the National Agriculture Statistics Service in 1998, there were 39 farms producing food carp, and 115 farms producing ornamental koi. While total farm sales valued at $5.2 million, food carp made up only $1.3 million farm sales, and koi farm sales valued at $3.9 million. In 2001 live carp exports were valued at $1.8 million.

KHV was first detected 1998 in July. Although no significant outbreaks have occurred, the disease has been diagnosed through routine diagnostics in 14 states. A total of 235 tests revealed only 48 positives. No detections were made in wild carp.

Since KHV is not an OIE list disease and to our knowledge has not caused a significant problem to our industry, this disease is not regulated in the U.S. At this time, there is no nationwide surveillance effort for KHV in the U.S., and any testing done is primarily diagnostic work on suspect cases or part of a voluntary certification/inspection for exports.

Rapporteur's Notes

Ron Hedrick. Many positives were from California. Very few laboratories are testing for KHV; the University of Georgia is doing PCR testing.

Andy Goodwin, Florida and Texas are just starting testing.

Israel

Avi Eldar, Israel

Only a verbal update was provided.

Rapporteur's Notes

Adrian Barnes. **Q:** Was the transmission of the virus by fish?
A: Yes. Also mechanical transmission. Between small producers it is difficult to control. Also in one case where a scientist was carrying out transmission experiments in carp.

Adrian Barnes. **Q:** 99% of the koi grown in Israel are for export. Were you exporting the virus?
A: Probably, but we were unaware of it.

Keith Davenport. **Q:** Do you think KHV has spread to the near East?
A: No, mainly because there is not much carp culture there.

Rapporteur's Notes - Final Discussion at end of Day 1

Keith Davenport. Q: **Is "naturally immune fish" a valid term?**

Mordi Haimi. **A:** We cannot disinfect ponds or stop birds moving between ponds. So we expose fish to a virulent virus, the fish spawn in February, using naturally resistant broodstock. The larvae are transferred to mud ponds for four months - the temperature could be 30°C. In July, fish 10g or greater are exposed to the virus under controlled conditions: they are transferred to concrete ponds at water temperature of approximately 21°C, and cohabited with KHV-infected fish obtained from a research station four days post experimental exposure to the virus. After five days the fish are moved to mud ponds at 30°C. In autumn (around October) there may be many mortalities if the initial exposure was poor, but if the exposure was good, there will be fewer mortalities. The fish are challenged again with KHV-infected fish in winter (January). The KHV-infected fish obtained from the research station are checked by PCR to confirm they are infected, and any dead fish are checked by PCR. The process takes 14 months, then the fish are sent for on-growing.

Trevor Hastings. **Q:** Is there any programme for producing genetically resistant fish?
Mordi Haimi. **A:** There is a carp that is resistant to KHV but it is not tasty and stops growing at 200g. One hybrid (C.carpio x Carassius) is also resistant.

Trevor Hastings. **Q:** Any resistant koi?
Mordi Haimi. **A:** Need to look at genes of resistant fish but work not done yet.

Peter Dixon. **Q:** You said your brood stock were naturally resistant. Is that an age-related resistance, or are they survivors of infection and immune?
Mordi Haimi. **A:** The broodstock were previously exposed, and are survivors of infection. They are stripped twice a year, and very few out of hundreds of brood fish die.

Peter Dixon. **Q:** A question to you all. Is there an age-related resistance? What is the upper age range of susceptibility?
Sven Bergmann. **A:** Three-summer carp can get infected.

Ron Hedrick. **Q:** Are there mortalities in this process of producing naturally resistant fish?
Mordi Haimi. **A:** Yes, but it is variable. This could be because of various factors such as temperature.

Ron Hedrick. **Q:** Is virus persisting in the fish, or are they being re-infected from an external source?.
Mordi Haimi. **A:** We believe virus persists in the environment (earth ponds) and when temperatures fall mortalities occur.

Chris Seagrave. **Q:** Can fish shed virus after the induction of natural immunity ?
Mordi Haimi. **A:** No. Both susceptible and resistant fish are sent to customers, but we get no reports of mortality. If we co-habit susceptible and resistant fish at a permissive temperature there are no mortalities.

Chris Seagrave. Anecdotal evidence suggests that if susceptible fish are mixed with Israeli fish, the susceptible fish will die. Has anyone else heard this ?

Keith Davenport. That is a question for the scientists to answer. It is a policy issue as well.

Mordi Haimi. There is a problem of terminology. "Resistant fish" must see the virus two times, and see permissive temperatures. Fish infected under other conditions would be dangerous.

Markus Biffar. It is important to find carrier fish. I do not agree with the safety of "naturally resistant" fish.

Manfred Neukirch. **Q:** What is the permissive temperature limit for infection ?
Mordi Haimi. **A:** About 30°C.

Manfred Neukirch. *In vitro* the virus grows at 30°C.

Ron Hedrick. 30°C is not permissive in KF-1 cells. The lower limit of replication is about 10°C.

Manfred Neukirch. We tried 28, 30 and 32°C. The virus grew at 30 but not 32°C.

Oran Gilad. The virus kills fish at 28°C, but it does not grow *in vitro* at 30°C. In ponds there will be fluctuations between day and night, e.g. 28-32°C, which could help virus to replicate in fish.

The chairman asked for more explanation of the effect of point mutations in the virus genome.

Ron Hedrick. A single point mutation could affect virulence. In my talk I did not mean to imply that a single point mutation did cause the differences. If the attenuated virus was replicating in a fish which was not showing disease, it could show serologically as KHV antibody-positive fish.

Keith Davenport. **Q:** How can we disinfect, or deal with infected fish?

Chris Walster. **A:** The virus does not last long outside the fish in water, although it seems to persist in mud ponds. **Q:** Could it survive in organic matter in facilities? Could there be re-infection from organics as well as carrier fish?

Moshe Kotler. **A:** The virus can survive for between 8 and 20 hours in water.

Mordi Haimi. **Q:** Where is the virus in mud ponds – is it in animals?

Ron Hedrick. **Q:** Are the ponds left fallow then restocked in Israel? In California the ponds are drained, dried then refilled.

Mordi Haimi. **A:** The ponds are drained, dried then limed. But not everything is disinfected, e.g. nets are not disinfected and there is no change of clothing between different areas.

Andy Goodwin. **Q:** Are there mortalities in spring when the water temperatures are permissive?
Mordi Haimi. **A:** The are no mortalities. Fry in the mud ponds cannot be infected naturally but experimentally the fish are susceptible.

Andy Goodwin. That implies the virus is not present in the ponds.

Mordi Haimi. **A:** I can't really answer that.

Chris Seagrave.**Q:** What is the best method for disinfection of ponds?
Barry Hill. **A:** If the ponds are treated in the same way as for e.g. SVC, then KHV would be destroyed.

Chris Seagrave.**Q:** How long will the virus survive in ponds?
Barry Hill. **A:** Research is needed.

Manfred Neukirch. We did some tests for survival at different temperatures using virus in tissue culture supernatant. Virus survived for up to 50 days at 4°C, up to 30 days at 10°C, up to 12 days at 25°C and up to 5 days at 30°C.
Oren Gilad : I do not think it is as straightforward as one single trigger to induce the disease. We have to consider the inter-relationship of Host-Pathogen-Environment.

Adrian Barnes. **Q:** Young fry in ponds do not get infected but fish exposed once to the virus may be infected in the autumn. What is the source of the infection?
Avi Eldar. **A:** The fish are carriers for 12 - 14 months.

Edward Branson. **Q:** Is there maternal immunity?
Ian Bricknell. **A:** Maternal immunity lasts about 4 days in carp.

Keith Davenport. **Q:** How can we detect carrier fish?
Ellen Ariel. **A:** Firstly, provoke the virus at the permissive temperature. Secondly, can antibody be detected at the permissive temperature?
Rudolf Hoffmann. **A:** You could cohabit sentinel fish with test fish for three weeks and see whether they become infected.

Keith Davenport. **Q:** How do you know what a naïve fish is? Is such co-habitation an animal experiment?
Rudolf Hoffmann. **A:** Yes, it is an experiment.

Oran Gilad. **A:** The seller could quarantine the fish.

Barry Hill. **Q:** Can you trust the seller? It needs third-party validation.

Avi Eldar. No-one can properly check the breeder.

Oran Gilad. The breeder should be responsible.

Mordi Haimi. This is very complicated - only some pieces of the jigsaw can be seen. We must focus on the fish and produce a resistant population; it doesn't matter if they are carriers or not.

Richard Cowan. I would be alarmed by this approach. Even with quarantine we could still get disease from illegal imports of wild fish. It would be best to start with a more Draconian policy and then relax it.

Moshe Kotler. Take the example for chicken farming: all chickens are immunised against Newcastle disease virus. Fish breeders should do the same for KHV.

Trevor Hastings. Avoidance would be a Draconian measure. Make sure eggs are virus-free by disinfection and rear the fish in truly virus-free systems. Rely on biosecurity. One farm in Israel is already trying this approach.

Moshe Kotler. No disease has been eradicated by vaccination - it is a medium term strategy. Use it for a limited time and then create a virus-free environment.

Neil Auchterlonie. Our concerns must be for wild carp as well as ornamentals.

Ellen Ariel. I agree. The situation in Japan could occur here in common carp, and carp farming is an important industry.

Adrian Barnes. If we concentrate on the fish, the assumption is that the virus will spread world-wide.

Markus Biffar. The virus is world-wide. But we need to know which fish are carriers. We need to keep the virus levels low, but without Draconian measures. I don't see a realistic chance to get rid of the virus.

Keith Davenport. There is a danger to native populations and Government agencies should not allow ornamental fish to be introduced into fisheries.

Ellen Ariel. I don't know how far the authorities can go with ornamental fish. It is different in Japan where ornamental and food fish are dealt with in the same way. With notifiable viruses we know where the virus is and we don't export from infected areas; we can then go for eradication. How can we protect the common carp industry?

Richard Cowan. I agree about not introducing ornamental fish into fisheries, but it does happen. Hobbyists and others put fish into ponds. In the UK there is a correlation between the presence of ornamentals in fisheries and their close proximity to roads.

Moshe Kotler. It is easier to immunise common carp than koi. Protection is obtained following putting fish into a tank containing the virus for 15-40 minutes.

Marc Engelsma. **Q:** What is the difference between the immune systems of common carp and koi?
Moshe Kotler. **A:** The common carp has a better immune system.

Chris Seagrave. Two groups of people are not represented at this workshop: 1) carp farmers in the developing world and 2) domestic koi hobbyists and carp fisheries. It is outrageous to suggest that we should not respect their interests.

Barry Hill. It has been said that populations are at risk, but the virus has been in Europe for some years. Why has there been no major disease outbreak in Europe in wild fish like in Japan? An epidemiological investigation is needed.

Adrian Barnes. We need to look at water temperatures.

David Stone. **Q:** This is a question for Pat Smith or Richard Cowan. Could fish be challenged with attenuated virus as a vaccine in the UK? If not, we should not allow these fish in.
Pat Smith. **A:** No.

Keith Davenport. **Q:** Have these "vaccines" been approved by the authorities in Israel?
Moshe Kotler. **A:** No.

Avi Eldar. No fish treated with attenuated virus are exported.

Richard Cowan. Firstly, as a question of risk, I am not satisfied with the risk. Secondly, from an ethical point of view I am uneasy about importing fish that have gone through a procedure that

we consider unethical. The second point is not for governments; therefore we should rely on the risks.

Barry Hill. If the unlicensed live vaccine were used in Israel and the fish were to have a resulting persistent infection with the attenuated virus, it is questionable that such vaccinated fish should be allowed to be imported into the EU where all vaccines have to meet rigorous licensing conditions.

David Stone. Both the attenuated vaccine and the use of live virus should be treated the same and both not allowed in.

Avi Eldar. Both are different. The procedure with the wild-type virus should be allowed.

Ellen Ariel. The risks for both procedures are the same.

DAY 2 - Presentations

KHV – Impacts and research priorities - an industry perspective

Keith Davenport

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Background

Keeping ornamental fish is a popular hobby worldwide. Estimates indicate that 3.5 million UK households keep fish either in aquaria or ponds. In the US the figure is 10-12 million. Koi (a strain of *Cyprinus carpio)* and goldfish (*Carassius auratus)* are the specimens of choice with which domestic garden ponds are stocked. There are an estimated 2 million garden ponds in the UK and a similar number in the US.

The industry employs significant numbers of people around the world. In total (including those working in retail, outlets, manufacturers of dry goods and the like) , though there are no accurate data, the number employed in the UK may be as high as 10,000 and in the EU 40,000. It follows that if there are no fish to sell then there is no industry or employment.

Impacts of KHV

It is impossible to list all the impacts. But certainly there has been some reduction in sales of koi because of lack of confidence on the behalf of importers and the buying public. There has been some element of buying preferences switching to goldfish, however these have lower sale values and the additional equipment sold for their maintenance tends to be less valuable. The industry recognises that goldfish are not immune from other specific herpes viruses and so lessons learnt from KHV could help avoid the possibility of significant future problems.

Imports of live freshwater ornamental fish to UK 1994-2003*

Year	**Israel**		**Japan**	
	Freight tonnes**	£ millions***	Freight tonnes**	£ millions***
1994	256	2.557	40	1.474
1996	265	2.039	56	1.721
1997	242	2.030	151	1.827
1998	221	1.566	90	2.273
1999	157	1.084	93	2.011
2000	139	0.971	86	2.907
2001	98	0.787	117	2.753

2002	125	0.893	139	3.080
~2003	166	1.118	139	2.763

* Derived from HM Customs data Com Code 030110100 ** Unless packing technology changes very significantly (which it hasn't) or the size profile of fish transported alters freight weight is a good indicator of trade volume*** monetary values of imports should be considered with caution as they are very dependant on fish prices, freight rates (which are included in the import value used for these statistics) and currency fluctuations. ~January to November only.

There is clear evidence for the UK and to a lesser extent in the EU of importers "buying around the problem" since it was first recognised in Israel in 1998. Israel exports not just koi, but significant quantities of goldfish and tropical aquarium fish species. Japan has been one of the major alternatives sources to which importers in the UK and other countries turned.

Additionally, to avoid KHV many importers have implemented at least some of the suggestion actions included in the OATA Koi Herpes Virus - KHV document released in December 2001.

Research priorities

For practical and commercial reasons the ornamental industry would propose the following research priorities. In all instances projects that deliver information that can be rapidly disseminated and concern the prevention, avoidance, practical management, or eradication of the virus from populations of fish or sites should be given priority.

Detection

Are we dealing with one or a number of diseases? Are KHV, CGNV and MMKC all the same virus?

Development of a detection protocol that can detect even latent viral populations preferably using non-lethal sampling techniques. The probability of false negatives should be quantified and this data should be made available at the time of each test so the associated risks can be assessed and management decisions made.

Vaccines

Development of a vaccine(s) which will be effective and permit detection methods to distinguish vaccinates from fish infected with wild type virus.

If vaccines are developed using attenuated virus strains then the mutations must be irreversible and demonstrably so to the standards required by the veterinary medicine authorities in the importing country.

Epidemiology

Development of clear ideas on the transmission and transmissibility of KHV. Investigation of factors other than temperature that precipitate frank disease.

The role (and any potential risks) that resistant strains of carp or other natural immunity may play in managing the disease caused by KHV.

Research that will answer questions such as is virulent KHV the result of a species jump?, a mutation of a previously benign virus? , koi being rear in new areas?, changing climatic conditions? Identifying farms or areas currently infected will inform management decisions answer to these questions will inform industry strategy in the future.

Keith Davenport, Presentation 8 – Rapporteur's Notes

Richard Cowan **Q:** Why has there not been a more significant move to breeding koi in the UK, particularly from imported eggs ?
A: Largely because koi grow faster at warmer temperatures. But UK koi production is increasing with an estimated 4 million koi bred each year.

Trevor Hastings : There is much less risk of importing disease with imported eggs. This can provide a good chance of breaking the infection cycle. Disinfection of eggs is very straightforward with well proven protocols available. Research is needed to look at vertical transmission of the virus but it is unlikely that this is a vehicle for transmission.

Adrian Barnes : Provided the history of his Pisces site, set up in the early '90s, using warm water from a power station outfall – I do not believe it will be easy to eliminate KHV without dramatic improvements in auditing and disease control procedures at export and import sites. Legislative controls in producer countries need to be tightened also. At the moment there are two blocks of producers, a large block that are producing KHV positive fish and a smaller block that are producing KHV negative fish. A decision is needed on import into the EU of fish that are quite probably KHV positive and the accompanying ethical considerations. The Professional Koi Dealers Association have been providing facilities for their members to voluntarily quarantine koi that they import into the UK.

Keith Davenport : OATA have 6-7 years experience of trace ability (auditing) and certification that has been applied in the Marine Aquarium Industry. Good practice at source is important and quarantine of imported fish also. I would like to see the OATA protocol on bio-security applied to all koi imports.

Adrian Barnes **Q.** How many fish are reaching dealers that have not been through quarantine ?
A. This is a discussion that can go round and round and this problem area will certainly be discussed in working group 1 later in the day.

Peter Dixon : A response from a scientific perspective. Research will be directed at investigating methods to detect latent KHV. But as with every virus test there is always a likelihood of false negatives. A test giving 100% certainty of absence of the disease agent is impossible. AIDS/HIV is an example where millions of dollars have been spent on trying to develop an infallible test without success. It should also be emphasised that virus taxonomy, the type of virus involved, is important as this can assist the direction of future research.

A regulatory perspective.

Barry Hill, CEFAS Weymouth. E-mail: b.j.hill@cefas.co.uk

Legislation specifically intended to prevent and control fish diseases has existed in Great Britain (GB) for almost 70 years following the introduction of The Diseases of Fish Act in 1937. This legislation provided the enforcing authorities with a wide range of powers and established certain legal requirements for fisheries owners, fish farmers and fish importers. The main provisions of the Act included restrictions on importation of live fish and eggs into GB, preliminary precautions to be taken on suspicion of a notifiable disease, powers of entry onto land to inspect waters therein or to take samples for examination, and powers to designate any disease as notifiable, publicly designate any waters or area bound to be infected by notifiable disease, prohibit or regulate the transport of live fish, eggs or feed from that area, and order the removal of dead and dying fish and their disposal. The GB national legislation has also provided strong measures for official control of any outbreaks of serious (notifiable) fish diseases within the country to prevent further spread and these powers have been used extensively over many years to ensure the nation maintains its high fish health status. However, this strong package of national measures was amended in 1993 to comply with the requirements of EU policy on free movement of goods, including live animals, between all EU Member States and on control of aquaculture animal diseases (Directives 91/67/EEC and 93/53/EEC).

Under the EU fish health regime, the entire territory of GB is classed as an approved zone for both VHS and IHN and is therefore given protection against introduction of these diseases from other Member States and third-countries. Additional protective measures have also been granted to GB by the European Commission for certain other diseases, including SVC of carp and Gyrodactylosis (*G. salaris*) of Atlantic salmon, and additional health guarantees with respect to BKD are currently undergoing the approval process. However, there has been no move towards making KHV disease notifiable in GB under the national legislation or to adding it to the list of diseases covered by the EU legislation including those in Commission Decision 2003/858/EEC that lays down the health certification requirements for imports of live fish and products into the EU from third-countries from 1 May 2004. This has primarily been due to KHV disease being seen as a problem limited to the ornamental koi carp trade and one which could be left to the industry to manage for itself e.g. by application of the protective guidelines issued by OATA to its members. The recent reports of cases of KHV disease associated with widespread high mortalities in farms producing carp for food in Indonesia and Japan and its detection in wild carp populations in Germany and the UK could cause this view to change and the disease to be subjected to regulatory control, particularly with respect to imports of live fish into the EU. Until then, under the harmonised rules, koi carp can be imported into any country of the EU and/or be transferred from one Member State to another without any requirement for certification of freedom from KHV. However, if it becomes seen as too high a threat then national or EU restrictions could be introduced rapidly as an emergency safeguard measure.

The right of a country to restrict trade when necessary to protect human, animal or plant life is recognised in the rules of the World Trade Organisation (WTO) under the "Agreement on the Application of Sanitary and Phytosanitary Measures" (SPS Agreement). However this requires that the measures are not applied in a manner that unjustifiably discriminates between countries with the same health status and are not applied as a disguised restriction on trade or health. One of the main aims of the SPS Agreement is to preserve the sovereign right of national governments to establish the level of animal health protection that they consider appropriate, whilst ensuring that this right is not used for trade protection purposes. Essentially, two options are available to WTO member countries to provide a scientific justification for an import health requirement. The first, and the one encouraged by WTO, is for countries to base their import health requirements on the standards, guidelines and recommendations issued by the Office International des Epizooties (OIE) as the World Organisation for Animal Health. Where these do not exist for a particular disease (e.g. one which is not listed by OIE), or in cases where a government chooses to apply stricter measures to protect itself, the importing country must be able to demonstrate that its measure is based on a full scientific assessment of the potential health risks (i.e. an import risk analysis).

The OIE standards and guidelines for fish are laid down in the Aquatic Animal Health Code (currently the 6th edition published in 2003) and in the Manual of Diagnostic Tests for Aquatic Animal Diseases (currently the 4th edition published in 2003). The principal aim of the Code and the Manual is to facilitate safe international trade in aquatic animals and their products by providing detailed guidance for disease surveillance, disease occurrence reporting and the requirements of both exporting and importing countries to avoid the risk of spreading the OIE-listed aquatic animal diseases. A priority function of the OIE is to inform Governments about the occurrence of important animal diseases and changes in their distribution worldwide. The provision of accurate, complete and timely information on aquatic animal health status generates increased international confidence in the disease regulatory systems operating in a particular exporting country and assists importing countries in assessing the risk of a proposed importation of live aquatic animals or their products. The urgency of dispatching information varies according to the classification of the disease. Recently, OIE has seen an increasing need to take into account the speed of spread of a disease (and/or its zoonotic importance), and to link these criteria to the reporting procedures. The primary purpose of listing a disease is for the OIE to collate and publish information on its occurrence worldwide and for exporting countries to make details of their disease status available to trading partners. It is to be welcomed that despite KHV diseases not being an OIE-listed disease, both Indonesia and Japan quickly reported their outbreaks to OIE thus ensuring wide publicity and 'transparency' regarding the occurrence of a serious disease that could affect their trading partners.

There is also recognition by OIE that it is important to have a rapid system for categorising new emerging diseases and to re-categorise other diseases that have assumed greater or less epidemiological importance. New criteria for listing an aquatic animal disease were adopted by OIE in May 2003 and must be used in the future by member countries to support any proposals for removing or adding diseases to the OIE list. So far, no proposal has been submitted for listing KHV disease using this procedure. The disease listing criteria will be explained.

Barry Hill, Presentation 9 – Rapporteur's Notes

Ed Branson **Q.** What measures can be taken when a new disease appears within the EU ?
A. New legislation is being drafted that will introduce a fast-track mechanism when a disease outbreak or major fish kill occurs. All movements from the affected region would be stopped until more was known about the disease. However, it may be 2 years before this legislation is implemented.

Discussions following working groups– Rapporteur's Notes

[The video record of the questions and answer sessions following Group 1 and 2 presentations was not recorded, and the notes recorded are therefore the main points only.]

Working group 1 - presentation by Adrian Barnes

Questions to be addressed: What are the tools required and criteria needed to substantiate KHV freedom at a site level to enable safe trade to continue? How can these approaches be used to contribute to OATA current best practice?

Main points made in the presentation were as follows:

Preference is to achieve KHV-free supply chains

- **Development of self-audit process**
- Recommended diagnostic tools
- Development of agreed Code of Practice
- Development and promotion of self-audit process
- List of self-auditing compliant sites
- List of compliant importers

- **Diagnostic tools**
- What tests are applicable?
- Decide on which fish groups to test and where (exporter/ importer)
- Develop reliable tests
- PCR
- Serology (KHV-specific antibody test)
- Good stress test protocol

- **Develop a Code of Practice**
- The Code of Practice will be developed and published and distributed to all attendees within two weeks
- Development and promotion of self-audit process
- Self-assessment questionnaire to producers within one month
- Promote recognition of audit process
- Auditing health status of producers
- Transparency of health records/testing
- Traceability of stock

- On-going education of farmers/ producers
- List of self-auditing compliant sites
- Publication of KHV test results as they become available

- **List of Compliant Importers**
- Isolation of new imported stock
- Minimum of 14 days between 23—28°C
- Combine with antibody test?

- **Overview**
- This is an interim plan to be refined and adapted to meet the target of allowing the industry to operate in a transparent and traceable supply chain to enable the consumer to make informed purchasing choices.

…and all the koi lived happily ever after!

Questions:

Trevor Hastings : Suggested that external audits of production sites should be considered and was surprised that it was thought that a code of practice could be produced in such a short time !

Working group 2 - presentation by Ron Hedrick

Questions to be addressed: Standardization of detection procedures - can we agree on what are the best methods and how they can be applied? What new approaches are needed? (***Ron Hedrick***)

Points from working group 2 :

Screening for virus carriers

- SCREENING of CARP STOCKS for KHV – best methods currently available
- PCR - with confirmation by sequencing
- ELISA for KHV antibody – but confirmation method not identified - information gap
- ELISA for antigen - not sure how sensitive this would be – information gap
- PCR or presence of antibody – apply both tests but either giving a positive is a presumptive KHV
- Possibility of detecting KHV in circulating leucocytes & macrophages – may be a more sensitive method - again a gap in knowledge
- KHV Latency – may be important – info. gap
- Tests on Broodstock - Non-lethal testing important 1) non-lethal PCR ? 2) Antibody
- Presence of yolk-sac antibody ? - info. Gap
- Age – ca.4 months or older PCR only - at permissive temperature 18C to 28C for 4 weeks (3 weeks as a very minimum ?)
- Multiple year classes – test a group of each class with PCR and/or antibody test on appropriate age fish
- Sample size - minimum 150 but not below 30 for a particular year class Broodstock – Antibody test on a minimum of 30.
- Tissues – Gill, Brain, kidney and intestine. Ability of detecting KHV DNA in brain tissue and presence of virus in intestine of carriers - Info.gap
- Pools - all tissues from maximum 5 fish

Standardisation

- Duplicate set of samples to be taken – to enable separate confirmation of a positive – e.g. A and B sample (as in drug testing)

- INTERIM POSITION – Use the best, most sensitive, most robust, single-round PCR – all published PCRs currently acceptable but need to ring trial PCRs around a network of labs
- Would hope to move to more sensitive e.g. nested PCR when these can be validated
- Need to push toward publication of PCR based on sequence from known genes

Diagnostic test for presence of clinical disease

- Presumptive diagnosis - Gross pathology / Histopathology / Virus isolation / PCR
- CONFIRMATION - Virus i.d.
- IFAT or similar
- PCR - sequence PCR products or use other confirmation method for the product/amplicon (e.g. oligonucleotide probe)
- Immunohistochemical confirmation of histopathology findings

Reagents

- Antibody ELISA – commercially available anti-carp IgM ?
- Need for standardisation of the Antibody ELISA
- Cell lines – 3 available KF-1 (USA), CCB (Germany) , KFC (Israel) – is the KFC an established cell line ?
- ALL METHODS and reagents need to be standardised and proficiency testing performed – ring trial around participating labs.

Questions:

Barry Hill **Q.** Why test (virus screen) 150 fish ? You need to know the prevalence of KHV infection in a carrier population to determine the correct sample size. You may not need to screen as many as 150 !
A. Agreed – and prevalence of antibody positive fish is also important.

Keith Davenport **Q.** Is the working group still recommending 2 weeks at 23-28 degC as a minimum quarantine requirement.
A. Yes – as the very minimum but better if a longer period is used.

Working group 3 – presentation by Barry Hill

Questions to be addressed: Identifying key information gaps. What research is underway and what is needed? Can efforts be coordinated and what funding sources are available?

Main points made in the presentation were as follows:

- Are wild carp populations at risk?
- If so under what conditions?
- Test wild fish in lab?
- Is it already endemic in the EU?

- Are survivors a risk?
- Controlled experiments with and without stress
- If no virus produced can assume they are safe

- Carrier detection methods to demonstrate absence

- Genetic/antigenic heterogenicity
- How many serotypes?

- Immune response to KHV
- Vaccine development
- Antibody testing for screening populations for previous infection

- Development of vaccine with marker
- Other control methods will not eliminate need
- Long-term R&D

Existing R&D
Molecular phylogenetics (CEFAS/UCAL/HU)
Detection Methods (CEFAS)
Carrier state (CEFAS/UCAL)
Antibody detection (CEFAS/HU/Munich)
Comparative pathogenesis (UCAL)
Pathogenesis (CEFAS/HU)
Non-specific immunity (CEFAS)
Survey of carp producers (CEFAS/Munich)
Survey of wild carp (Munich)
Vaccine production (HU)
Diagnostic kits for virus and for antibodies (HU)
Effect of temperature on virus multiplication and storage (Hanover)
Influence of pH on virus infectivity (Hanover)
Influence of flavenoids on in vitro multiplication (Hanover)

Questions:

Peter Dixon : Asked delegates to inform the group of any other areas of research on KHV at their laboratories not currently on the list drawn up by the working group. It is important to give some idea of finance and manpower input into the research.

Mark James: Suggested that delegates used the proforma circulated by FRM Ltd to list the work of their research groups.

Barry Hill had pointed out that mapping the distribution of KHV disease cases was important and that this should be based on confirmed cases and not rumour and hearsay.

Peter Dixon: Pointed out that there was an Email list server available to distribute information and details of how to access this would be sent to all delegates.

Keith Davenport : Confirmed that this was administered by OATA and they would be happy to continue the service.

Ron Hedrick – on the problem of convincing funding bodies that this is a serious disease. **Q.** What about listing the disease with OIE as a means of adding gravity to the disease problem ? Within OIE circles is it moving towards listing ?
A. It has not been formally presented to OIE as a major disease problem. But we can apply the new criteria for listing to KHV and see what happens ! However, one of the restricting factors to the listing of KHV is the lack of a suitable test to demonstrate absence of the virus. So, data on disease status from countries is unreliable because testing is unreliable. However, if KHV goes onto the OIE notifiable list then it will almost automatically go onto the EU list.

Trevor Hasting – comment in response to the working group not including genetic breeding for disease resistance on its research 'shopping list', mainly because it is a lengthy process. Genetic breeding for resistance to KHV should be considered. In salmon it has been a rapid process of producing families resistant to particular virus diseases and similar techniques may be applicable to carp.

Oren Gilad **Q.** Is there any money available that might pay for reagents used in collaborative EU research projects ?
A. No – but there may be other co-operative research programmes available, that are not part of the current framework 6 programme, where the US might obtain fuller funding.

Ellen Ariel **Q.** Can survivors of KHV infection be traded and is there any precedence for this with other diseases ?
A. No – there is no precedence for this. However, relaxing of the legislation may make it possible for certain products to be traded (e,g, fish fillets, dead fish) if it could be shown that there was no risk.

Concluding Discussion

Keith Way : Called for a steering group to be set-up to progress the areas of work identified by the three working groups.

Keith Davenport : Suggested that greater communication and discussion should be promoted and mentioned again the OATA E-mail list server.

Peter Dixon : A lot of momentum has been generated and we should not let it drift.

Keith Davenport : Agreed – need to set some targets and keep the momentum going.

Ron Hedrick **Q.** Is the information supplied by the diagnostic methods working group enough to launch a volunteer register ?
Keith Davenport **A.** Yes - we need to go forward. This is an important issue and we need to identify synergies between the three working groups. Some of the points may need refining because all of the group presentations were prepared in a hurry.

Ellen Ariel : Offered the experience of the Danish lab. to organise ring-testing of the methods identified but they would also require input from other laboratories.

Mark James : Defra is looking to this workshop to advise on targeting further funding. There is a possibility that Defra could potentially support a collaborative steering group and it should be noted that a steering effort is required.

Keith Davenport : Agreed - Perhaps an informal strategic group as an interim position before funding becomes available.

Adrian Barnes : Within the ornamental fish industry there are different levels of involvement with koi. We need to identify others in producer countries on the steering group and we need scientists also. I propose Keith Davenport as the chair of the group with volunteer representatives from each of the working groups.

Keith Davenport then drew the meeting to a close. He thanked everyone for attending and especially thanked those delegates that had travelled so far to attend the workshop. He also thanked Defra for sponsoring the workshop and Mark James for organising the event.

Acknowledgements

The organisers of the International Koi Herpesvirus Workshop; FRM Ltd, and the Ornamental and Aquatic Trade Association (OATA) would like to acknowledge Defra (Fish II) for financial support for the workshop. The rapporteurs were Kevin Denham, Peter Dixon, David Stone and Keith Way from the CEFAS Laboratory - Weymouth. We are indebted to Keith Davenport, Chief Executive of OATA for providing excellent Chairmanship. Mark James would like thank all those participants who contributed freely to the discussions and ensured the success of the workshop - and hopefully a more rapid solution to KHV.

ANNEX 1. KHV WORKSHOP - Attendees

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ANNEX 2. Additional Information/Discussion Papers Submitted

The present situation and the preventive infection of KHV by Nishikigoi (Koi) industry

Shunichi Yoshida, Chairman of Fish Disease Measures Japan Nishikigoi Shinkokai.

The Japanese Government reported the occurrence of Koi Herpesvirus (KHV) as required. However, we are afraid that there is a misunderstanding about the situation of Koi (Nishikigoi). According to Japanese Law, , infected fish are not allow to be moved, and must be destroyed. Therefore most fish were destroyed rather than dieing as a direct result of KHV infection. Japan has controlled infections more than other countries. KHV infected fish from Kasumigaura prefecture were transported to other prefectures and spread the disease. However, all KHV infected fish are reported and destroyed. The purpose of this law is to prevent the spreading of disease by destroying infected fish, to eradicate KHV, and prevent further infection of Nishikigoi.

It is important to note that most Japanese KHV disease reports did not distinguish between Common Carp and Koi clearly. In Japan, Koi refers to both Common Carp and Nishikigoi. This difference in interpretation may be the cause of misunderstanding. As such, we have investigated the situation ourselves. The result and the response are following:

Current Situation with Regard to Infection
There is infection reported for the movement of Common Carp, but no infection report for Nishikigoi movement.

Self-Imposed Movement Restrictions
Only Niigata prefecture has self-imposed movement restrictions. However, this restriction was imposed due to the infection of Common Carp., Nishikigoi were not infected by KHV. These movement restrictions will be lifted this month, as the last two inspections have been negative for KHV infestion.

Disease Management
Even in winter, Nishikigoi for export are maintained in temperature controlled ponds (at a temperature at which it is possible for KHV disease to develop). If the fish is infected, KHV appears , but at present, no infection appears from Koi in these conditioning ponds.
Almost all farms do not use water from rivers where there are Common Carp, to prevent invasion of parasites and other diseases.

Domestic Response to Prevent the Spread of the Disease
To prevent infection, all Japanese Style Koi Shows and Nishikigoi auctions were stopped after out break of KHV. We are recommending Koi keepers to quarantine Koi and most of retailers are testing fish with PCR.

The Export Situation
Only Koi that are from farms with negative PCR test results are exported. In addition, Koi must be isolated in water at 21-23□ for two to three weeks and display no unusual symptoms before they are send abroad. As a precaution, we recommend importing countries quarantine and test Koi.

Future Measures
We test our fish regularly with PCR and quarantine fish. In addition, we are currently discussing with Government the possibility of authorization of disease free farms and the issue of health certificates to permit export.

KHV - A challenge to the future of Koi

Adrian Barnes, Chairman of the Professional Koi Dealers Association.

Introduction
I am the owner of Crownpark Koi, exclusive importers of Konishi Koi Farms Koi to the UK and also a member, and at present, Chairman of the PKDA (Professional Koi Dealers Association). It was as PKDA Chairman that I was asked to relay our member's experience of KHV in the UK Koi fraternity. KHV was not new to me although at the time of my initial meeting with this virus it did not have a name.

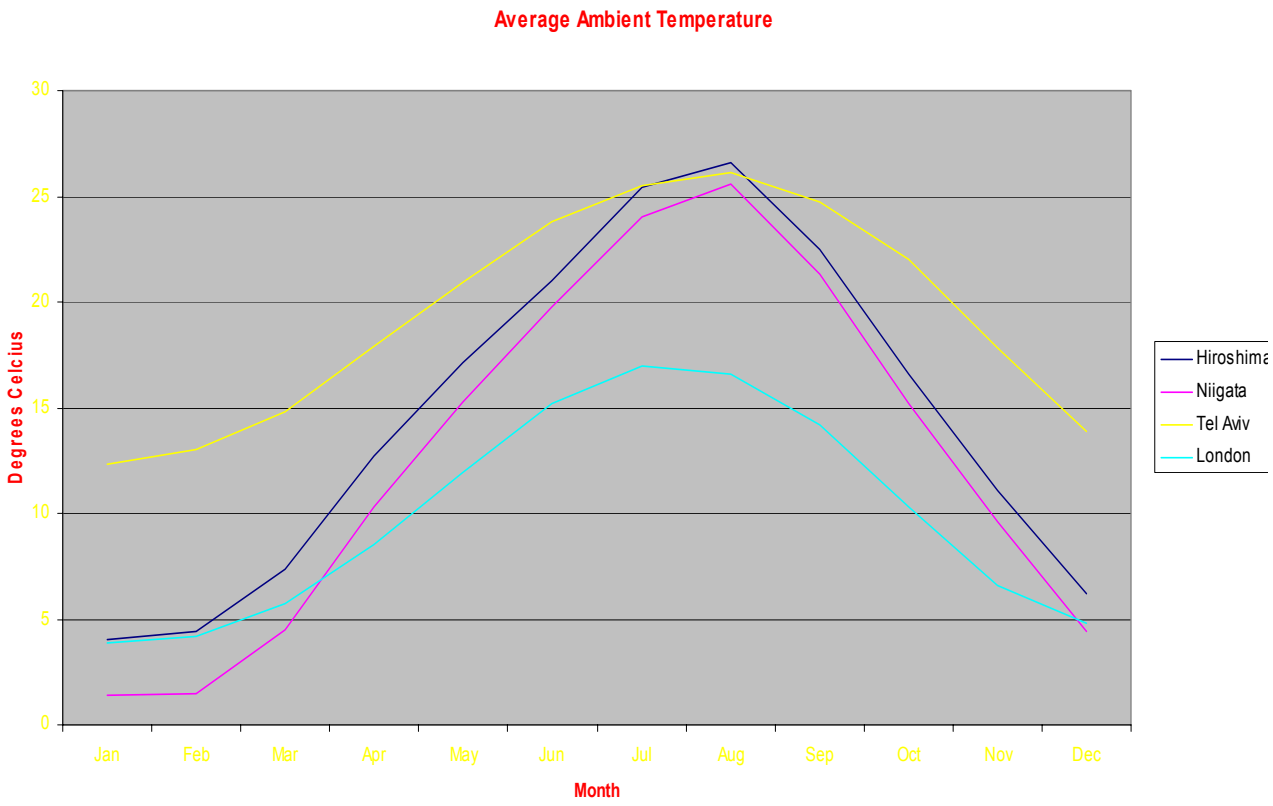
In August of 1996 the KHV incident occurred on my farm in Derbyshire. This was a fish production facility utilizing the waste hot water from a power station to grow fish. Water temperatures were from 24 – 28 Celsius year round. On the farm many species of fish were grown in polyculture (in the same tanks) in addition to the 17 tons of common carp and koi. These included 300 tons of Tilapia, goldfish, sturgeon and orfe.

I first noticed that the behavior of the koi and carp was unusual. They were lethargic and stood in the water column unable to swim against the flow. Microscopic examination of these fish showed a high level of ectoparasites and treatment was made against this in some of the tanks. Within the next two days mortalities began with the carp showing high levels of mucous loss on the skin and gills. The skin of these koi and carp seemed to be "stripping off". Both in treated and untreated tanks, the mortalities were only of the carp – none of the other species of fish showed any signs of infection – not even with ectoparasites. For the next ten days all of the staff of the farm were occupied with the continuous removal of dead carp. Within a week only a small percentage of survivors remained. This incident occurred exactly two weeks after the addition of new koi from an outside source. Although unknown as KHV at the time, this pattern of infection, and the analysis made by Stirling University at the time indicated an immuno-suppresory syndrome likely to be virus. The temperature of the water, timing after the new koi were added, together with the skin loss and speed of mortalities became the hallmarks of KHV virus when it occurs.

In 1998 the virus was given a name and a test was developed that, to this day, is considered not to be an accurate indicator of viral presence. The established PCR test does not identify KHV unless the virus is active and is thus unreliable or inefficient in detecting koi that look healthy but that are in fact viral carriers. This means that the only real method of knowing if a koi is a potential risk is after it has infected a naïve koi population with the virus and the mass mortality occurs.

Work on KHV has been performed in Israel where the problem has been recognized since 1998. Two of the lectures in the recent Tokyo summit Tokyo were given on this – one by Mag Noy and the other by Moshe Kotler, the researcher on a KHV vaccine. It is a stated opinion of the Israel's Mag Noy producers that the KHV virus was imported into Israel from the UK on equipment in late 1996 and only started to be active in 1998.

The dealers and hobbyists in the UK were witnessing mortalities in populations of koi that had fish added to from Israel as early as 1996, though the ability to pinpoint the origin without any traceability procedures means this is not definite.
Where we agree even less to the stated explanation of Mag Noy is that the sale of koi from Israel from 1998 to 2001 and of NIS (naturally immune fish) from 2001 onwards did not continue to carry and spread the virus. Too many instances of KHV relating to the recent addition of Israeli koi to the ponds of our members customers create a powerful body of circumstantial, even if this is not foolproof evidence. Why has this not been a clear picture? The answer lies in the difference in the water temperatures in Israel and the EEC - the main koi market.



The water temperatures in the UK and Europe are normally below the critical temperature range of the virus, (18-25 Celsius) except for some summers for a couple of months, or in artificially heated ponds. In this case the virus remains dormant after import.

Subsequent outbreak of the virus occurs when a certain threshold of temperature and in many cases a further environmental trigger are reached. An increasing number of "pond wipe-outs" were reported to koi dealers during each summer from the mid 1990's, and peaked in the summer of 2003, which was unusually warm.

The PKDA predicted that the total loss of koi collections by hobbyists would reduce the number of hobbyists, and that the short term gain of selling koi through the consolidation chain, with a potential KHV infection would reduce the size of the industry for all involved.

The PKDA's Code of Practice requires fundamentally different practices to be performed by its members, than were and still are, a reality of the majority of the koi marketing chain. The majority of the ornamental fish imported into the EEC are distributed in their packs of origin directly to the retailers sale tanks, meaning that an immeasurable percentage of the fish reach hobbyists ponds within days of their import. The costs of koi sold through this system are, in general, cheaper at import rates and are maintained inexpensive by this method of distribution. This system would be acceptable if there was a system in place to check and only allow import of koi that had no potential to transmit KHV at the production farm. Dealers purchasing koi from more than one source take on the risk of cross infection even when sanitary procedures are in place. This makes it even more difficult to decide which supply is the real source of the virus. Since the majority of all the koi are imported is spring prior to the temperatures reaching the critical range, a KHV potential supply can reach mass distribution, without any incidents. It was thus a requirement of the Code of practice that all fish sold by PKDA members to hobbyists be after a quarantine period of three weeks from the importation of these koi. Unusual mortalities on any batch, it was agreed would be reported to the Association's health consultant so that a database could be maintained and used to indicate potential sources of infection though KHV.

This database has brought up hundreds of incidents that indicate the presence of KHV.

Since virus destroyed nearly all of the 17 tons of koi and carp stocks on my farm in Derbyshire in 1996 I have attempted time and time again to counteract the process of denial taken by the industry to the existence of KHV. The denial is perpetuated by collusion between the producers of potential KHV carrying stocks and the importers of these fish in the market countries.
The trade press are also party to this fiction by ignoring the existence of the virus. A good example of this is the publication in July 1999 simultaneously in a national Israel newspaper about the mass mortality of koi due to KHV on Kibbutz Gan Shmuel and an article in the UK Koi Carp magazine encouraging the hobbyists to by koi from this source.
At the start of this year there is now news of a vaccine that has been applied to many of Israel's koi, which will be sold in Europe. Having been the target for many years to the NIS (naturally immune) koi, the UK European and world koi market will be the experiment for the attenuated virus vaccinated koi. _The impact of KHV on the market has been to create two separate koi markets – one that is the cheaper, and what can be termed, the "annual" koi market where the purchasers are less interested in the long- term survival of these koi. The other market is the more expensive "pet" and show type koi (nishikigoi) where the longevity of the koi is important. It is in general the more expensive koi market that is really threatened by KHV.

We believe that if the koi trade in the UK and Europe is able to demonstrate audited procedures, together with a system of traceability and transparency, then it will be unreasonable of the EEC to instigate a ban on importation.

The research on vaccines and other treatments could obviously bring about a solution to this condition. However it is necessary to test these prior to mass application. An independent third party should perform this testing.

ANNEX 3. List of Powerpoint presentations available electronically

1. Initial Isolation and Characterization of a Herpes-like Virus (KHV) from Koi
2. Prevention of a mortal disease of carps induced by the carp interstitial nephritis and gill necrosis virus (CNGV)
3. Koi herpesvirus : Diagnostics and research at CEFAS Weymouth laboratory 2000 – 2003
4. KHV and possible existence of a carrier state
5. Detection and isolation of KHV in Continental Europe
6. Outbreak of disease causing mass mortality in koi and common carp (*Cyprinus carpio*) in Indonesia
7. Global distribution of KHV with particular reference to Europe
8. KHV – Impacts and research priorities - an industry perspective
9. A regulatory perspective
10. Working groups 1-3 - presentations by: Adrian Barnes, Ron Hedrick, Barry Hill

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