KOI HERPES VIRUS

(KHV)

December 2001
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INTRODUCTION

Koi Herpes Virus (KHV) has only been identified as a cause of koi and carp deaths over the last three years. Problems have been experienced by exporters, importers, retailers and hobbyists. This was made plain by responses to a fish health survey conducted by OATA in the UK during the late summer of 2001. Almost 50% of respondents had experienced koi or carp specific mortalities which had a rapid onset and high death rate.

Information is limited. Only a few research groups are studying the virus and the disease it causes. This report may seem long and complex. This is necessary to ensure that as full a picture of what is and isn’t known is provided. A shorter report would have made it necessary to abbreviate the description of the gaps in knowledge which could affect the judgements of risk and possibly the actions you take to limit the risk KHV poses to your business. This report should be read completely before any conclusions are drawn or decisions are made. We hope this report will help in choosing coldwater fish in the spring. Moreover, we hope it limits the entry of the virus into the supply chain, and by doing so helps restore confidence in the market.

The most detailed information on specific topics is included in Annexes at the back of the report. At the front is a brief overview of what is known about KHV and a list of the suggested actions made in the text of the main report.
SUMMARY OF SUGGESTED ACTIONS

This summary is provided as a concise reminder of the suggestions made in this report. In each case the reader should refer to the relevant section of the report to satisfy themselves that they understand the strengths and weaknesses of the information upon which it is based. This summary should not be read in isolation from the main text of the document.

Very careful questioning of sources of fish supply using the type of questions outlined in this report should be a first step in managing the risks presented by KHV. This might apply equally to importers (both wholesalers and consolidators) seeking assurances from exporters and to retailers seeking assurances from wholesalers or consolidators (page 22).

As the state of knowledge stands at the moment, a negative PCR (polymerase chain reaction) result must not be taken as giving any guarantees or assurances beyond the fact that the virus could not be found in a batch of fish at a particular time. This may mean little more than the fish were not ill at the time. No absolute claims to be “disease free” or have “virus free” status can or should be made on the basis of these tests (page 20).

The PCR test available at CEFAS may be used to help screen sources of supply. Care should be taken to understand when this test may be used and how any results may be interpreted (page 20).

Each batch of fish should be isolated completely. No transfer of fish, water or equipment should be permitted. If you require exporters to give assurances as part of the contract of supply, they may require that you provide evidence of effective isolation policy of new stocks and its effective practical application (page 23-24).

Basic records of water quality, mortalities and observations of fish stocks should be maintained and held for reference (page 24).

Fish should be subject to a period of “preventative acclimatisation/isolation” of at least 14 days, ideally longer at between 23 and 28°C. Any batch of fish in which signs of illness or disease and particularly showing mortalities similar to what this report has called the “calling card” of KHV should be subject to PCR tests. If these prove positive then that source of supply may be considered unsuitable until adequate remedial action has been undertaken (page 24).

Batches from major sources of supply might usefully be screened prior to the start of the next coldwater season. To be confident of the results of such screening, the isolation policy must be absolute. Given the apparent infectious nature of the virus in closed systems, no chances of cross contamination should be tolerated if the PCR results are to be useful (page 24).

All water should be discharged via the foul sewerage system (page 24).

No koi or other carp should be accepted from any unknown source, including retail customers, and returned to your holding systems (page 24).

The risk of losses may be reduced by importing smaller consignments more frequently than perhaps has been the custom in the past. However this may require more investment in isolation facilities (page 24).

Any claims to supply immunised fish or fish which are naturally immune should be accompanied by written assurances, as part of an enforceable contract of supply that these fish are not carriers of latent KHV
infections. Once again in the event of a claim you may justifiably be called upon to establish the efficiency of your isolation policy (page 24-25).

Properly supervised administration of correctly chosen antibiotics may enable some fish to survive outbreaks. However these may remain carriers of KHV (page 25).

Fish surviving outbreaks of KHV should be regarded as potential carriers of the disease. It may prove unwise to mix these fish with any other stocks. Members of the public presented with this situation may choose to dispose of the remaining stock and buy new fish or maintain a lower stocking (page 25-26).

Disinfection, following a suspected outbreak of KHV or indeed any serious disease, may be carried out using the techniques outlined in Annex B by MAFF (now DEFRA) for SVC. Further advice on this technique or alternatives is available from the Fish Health Inspectorate at CEFAS T: 01305 206673/4 or fishhealthinspectorate@cefas.co.uk (page 26).
SUMMARY OF FACTS

RECOGNISING KHV CALLING CARDS

KHV should be considered as a potential culprit (not the culprit) if:

- Only carp (*Cyprinus carpio*) including koi, ghosts and common varieties are affected and killed. Other species such as goldfish and grass carp in the same system remain unaffected and might even be kept in contact with koi to aid diagnosis of KHV.
- Mortalities occur at water temperature between 18-30 ºC. If during an outbreak the temperature rises above or falls below this range mortalities diminish or stop.
- Mortalities are very rapid. Seemingly healthy fish become ill and die in 24 to 48 hours.
- Very severe mortalities. 80-100% mortalities occur within 10 days of disease outbreak.

The clinical signs associated with KHV disease are generally very similar to those of many common bacterial infections and parasite infestations. The presence of KHV cannot be definitively determined purely by observing the fishes appearance or clinical signs alone.

CLINICAL AND PHYSICAL SIGNS OF KHV

Clinical and physical signs include:

- Bleeding from the gills
- White patches (where excess mucus has been produced or tissue has died) which may be small to very extensive on the gills
- Pale patches and blistering on the skin
- Sunken eyes
- Occasionally fish have shown signs of nervous problems (that is periods of inactivity followed by hyperactive behaviour triggered by a very small stimulant)

Secondary invasions of bacteria and parasites might be the most obvious problem on an individual fish, but these signs may obscure the damage caused by the virus.

UNDER THE MICROSCOPE

Damage to the gills may be seen by using wet preparations. You may also just be aware of large numbers of bacteria and parasites in the mucus. This is the limit of the investigations possible in even those retail outlets possessing and using a light microscope, and a diagnosis cannot be made on this information alone.

In heavily infected fish, scientific laboratories are able to prepare and stain very thin sections of tissue (this is known as histology) in which they may be able to see signs characteristic of KHV. Under extremely powerful electron microscopes individual virus particles can be seen.
POLYMERASE CHAIN REACTION - PCR

Polymerase chain reaction tests are the most accurate method available. They can only detect virus in fish showing clinical signs of the disease.

GEOGRAPHIC DISTRIBUTION

Since 1998 KHV has been found in the UK and on continental Europe, the USA and Israel. Rumours of its presence elsewhere such as Japan are rife, but remain to be proved.

KHV IN THE UK

Since 1998 there have been cases each summer.

Diagram 1: The significance of temperature in koi outbreaks
THE SIGNIFICANCE OF TEMPERATURE

At low temperatures, the body's immune system is virtually shut-down. Immune activity increases with temperature. The optimum temperature for the KHV virus lies between 18-27°C. This information is summarised in diagram 1.

Most mortalities occur between 22-27°C, being very much reduced below this and there being virtually no occurrence at 30°C or above.

The longer water temperature remains between 18-27°C, the more likely it is that an outbreak will occur, if the virus is present.
Fish that survive the disease may always remain as carriers in the population. These fish, though infected with the virus are not affected by it, but can release virus particles to infect other fish.

KHV cannot be treated directly.

**TREATMENT OF VIRUSES**

**NO** treatment exists for viral diseases in fish.

Preventative measures include:

- Asking for relevant information from exporters that is in writing and forms the basis of the conditions of sale.
- Isolation of new stock for at least 14 days between 18-27°C.
- Use of the PCR available at CEFAS to screen as appropriate sources of supply and fish sold.
RISK ANALYSIS

Risk analysis is an everyday occupation for all business men. Will we make a profit if we stock this or that product? What are the risks of spoilage and loss if we buy food from this or that source? In almost all cases decisions are made on the best information available: almost invariably there are gaps in that information about which judgements have to be made. Clearly the more definite and complete the information the more likely you are to be able to make a correct decision. Each business must make its own decisions based on experience and their own attitude to risk.

In this document OATA has tried to draw together the most recent accurate data on KHV. Indications of best practice guidelines reflect the balance between risk and trading from an overall industry position.

The first step of risk analysis is the identification for a process eg the import of live fish:

- What can go wrong
- The likelihood of the process going wrong
- The consequences of it going wrong (fish health, economic etc)
- How can the consequences be limited

In the past perceived risk or qualitative standards might have been an adequate basis upon which to make purchasing decisions; this might not be the case for the future.

Among the questions that require answers for KHV are:

- Which species are susceptible?
- Can the disease be recognised by the clinical signs that can be seen?
- Can the presence of a pathogen be determined accurately?
- Which tests give the most accurate reliable results?
- Does a positive detection test result indicate the presence of infective pathogens?
- Will pathogens survive transport?
- Will the pathogens survive being introduced into a new environment?
- Can pathogens survive in bacterial films on fish holding and filter systems or must they be on the host organism at all times?
- Does the presence of a pathogen indicate that an outbreak of disease will occur?
- What is an infective dose of pathogen? Is it the same in all conditions in all species?

We have tried to answer these questions, and more, as best current information allows. However we have not answered them in the order above but incorporated them under the three headings outlined below. Usually in risk analysis a fourth heading “Hazard communication” is used, it is hoped this document will, for the present, serve that purpose for KHV.

Risk Analysis can be broken into three phases:

- **Hazard identification**: what organisms pose a risk?
- **Risk assessment**: under what conditions will they pose a risk? How great is the risk?
- **Risk management**: what can be done to limit the risks?
HAZARD IDENTIFICATION

The hazard is the disease caused by the Koi Herpes Virus – KHV. It is not the first and will not be the last virus to cause difficulties for our industry.

RECENT HISTORY OF FISH VIRUSES IN THE ORNAMENTAL TRADE

Below are some examples of diseases caused by viruses that have caused problems for the industry over the last decade. A number of these have been described as “new”.

- UK - several times in the last decade Spring Viraemia of Carp (SVC) has caused difficulties for both the ornamental and angling industries.
- Taiwan - 1995 – 90% mortalities in a goldfish breeding establishment associated with the presence of a herpes like virus.
- Japan - 1996 – corona virus causing skin ulceration and high mortality in coloured carp.
- Korea summer 1998 to present - a new viral disease causing high mortalities. When tested against “Israel carp” 100% mortality resulted.
- Carp pox has caused unsightly growths on older fish and have been responsible for mortalities in fry. The technical name for the virus causing the pox is cyprinid herpes virus (CHV).

But it’s not just the ornamental fish industry affected by herpes virus. They have been causing difficulties from Holland to Hobart. In Holland a recent scientific poster outlined a herpes virus that infects eels and has been found on 20 occasions in the last 15 years. In France herpes is blamed for causing mass mortalities in scallops during September 2000. In Australia massive mortalities, in the wild, among pilchards was blamed on a herpes virus carried on frozen trash fish fed to cage reared tuna.

HERPES VIRUSES

The word “herpes” comes from the Greek to creep. This group of viruses received this name because they are able to creep into inaccessible places in the body and possibly become undetectable.

Herpes infections are found in a wide range of animals, apart from fish, including elephants, monkeys, dogs, cats, tortoises, parrots and man. Eight herpes viruses are known to infect man and the diseases they cause include cold sores, glandular fever, chicken pox and shingles.

In many of these infections the disease is recurrent, flaring up periodically when the person or animal is stressed or run down in some way. In between these disease episodes the virus creeps away, for instance the cold sore virus virtually disappears inside nerve complexes near the spine. Since they are inside the actual nerve cell the bodies defence mechanisms do not recognise them as a threat, and so
the virus is safe. In this phase of their lifecycle the virus is termed latent. In this latent phase they may enter the genetic material of the fish making them extremely difficult or impossible to detect.

Thus it appears in many instances once an animal is infected with a herpes virus, it is infected for life. Herpes infections are generally characterised as being:

- Chronic
- Latent
- Recurrent

At a recent meeting in Dublin, a scientist from Germany outlined an experiment in which carp surviving an outbreak of KHV were apparently able to infect a new batch of fish a year later!!

THE HISTORY OF KHV

ISRAEL

The first known outbreak was described at Magan Michael, Israel during spring 1998. The official authorities in Israel expressed the view that this outbreak was in some way associated with the illegal import of fish or fish farm equipment from elsewhere in Europe, possibly the UK.

Further outbreaks were confirmed during both the autumn of 1998 and spring 1999. During this period it is estimated that fish farmers lost 600 tonnes of common carp for the table, and $4 million worth of koi for export. It was not until 1999 that KHV was identified as the culprit, this information was then published in the scientific press in mid 2000.

Magan Michael farms a number of species in semi-saline water on the coast of Israel. Since the initial outbreak we believe all kibbutzim associated with Mag Noy (as of the start of this year) have had outbreaks of the disease. The most recent being Hazorea in April 2001.

Early in 2001 KHV was added to the diseases listed in the Israeli Animal Disease Ordinance. It is difficult to interpret what this means in practical terms, as it would appear all animal diseases should be reported to the official authorities in Israel. (It would however, appear to be justified to conclude that KHV was still causing widespread problems to farmers in Israel.) A recent article in the fish farming press reported a massive reduction in carp production. The official authorities in Israel’s Central Fish Health Laboratory commented “...the disease bearing the temporary name KHV continues to be found in Israel. We have learned to cope with it, and the annual yield has returned to what it was formerly.”

ELSEWHERE

Evidence suggests KHV was also found in the USA, widely in Europe in 1998 and 1999 and 2001. Rumours abound (though with no absolute proof) that KHV is present in Japan. There are indications that South Africa had problems with the virus in 1998.

Outside of Israel almost all known cases have occurred in ornamental fish in farms, wholesalers or retailers. However, at least one case on a German fish farm raising carp for food or angling has been reported.
There have been reports of incidences of the diseases in the early nineties, well before the Magan Michael outbreak. These comments are based on visual observations of disease outbreaks, that as outlined later in this report, can be misleading and cannot give a definite diagnosis. However techniques exist which may allow detection of the virus in preserved tissue samples taken at the time of these disease outbreaks about which there are suspicions. Considerable further research will be needed before we really know when KHV first reared its ugly head. For the time being the outbreak at Magan remains the first outbreak that has been conclusively identified.

**ARE WE SURE SCIENTISTS HAVE IDENTIFIED THE GUILTY VIRUS CORRECTLY?**

There are rules used by scientists to decide if a particular virus is causing a particular problem. In the case of KHV the following experiment was carried out and repeated:

- Tissue extracts were made from clearly diseased fish.
- Virus was then grown in cells from koi fins. Characteristic changes indicative of an infective virus were seen in these cell cultures.
- This material (including the viruses that had grown) was then injected into koi; 80 to 100% of those fish injected died.
- The same virus found in the original diseased fish was then found to be present in the injected fish.

As part of the process of making extracts from fish showing clear signs of the disease the material is passed through a very fine filter to exclude bacteria. Electron microscopy is used to identify that the virus particles present in both the original ill fish, and those infected by injection with extracts from them, are the same. Thus while it is not entirely impossible that the wrong organism is being blamed for the disease, it is highly unlikely.

**WHERE DO “NEW” VIRUSES COME FROM?**

These viruses are probably not new but have always been around. Viruses, like many bacteria and parasites, are opportunistic, normally they are present in small numbers and cause little or no problem.

However if conditions change they make take advantage of the situation, for instance:

- Viruses have been known to jump from one species to another, and even from a saltwater fish to a freshwater species. Harmless in their original host species, they become very virulent in the new one.
- Geographically separate strains of the same fish species may become immune or resistant to different bacteria and viruses. When mixed the result is that organisms that cause no problem to one strain, will cause disease in the other. A human example is the decimation of remote tribes by common diseases of western settlers in Africa and America.
Changes in husbandry conditions such as rises or falls in temperature or stocking density will change the types and level of stress the fish are subjected to, and may promote susceptibility to new diseases organisms.
RISK ASSESSMENT

UNCERTAINTY IS (ALMOST) THE ONLY CERTAINTY

KHV was only recognised as a disease three years ago. The virus responsible was only identified two years ago and the scientific paper announcing this to the world was only published last year. The biology of the virus is little understood, and thus solutions to the problems it can cause must, at this point be based on an incomplete data set.

We must reiterate that this document is what we believe to be, at the time of writing, the best available information known about the virus, and how it may be avoided or managed. Since the information is so incomplete, it is almost inevitable that advice about best practice will change with time. That said, universally accepted basic husbandry rules will always apply, and will always help prevent disease outbreaks.

The notes and recommendations in this report are based on the limited information presented. Any member choosing to act on the information included here must do so on that basis. No liability for loss occasioned to any business or person taking action or refraining from taking action, as a result of any material in this document can be accepted by the author or OATA Ltd.

Each business must ultimately make a choice of strategy based on its particular circumstances and attitude to risk.
RISK MANAGEMENT

DISEASES ALWAYS COST MONEY!!!! (EVEN IF YOU DON’T GET THEM)

Whether in costs of prevention or in dealing with the problems of an outbreak, it is an inescapable fact that diseases cost money. Each business has a choice of where it meets those costs, either in avoiding a disease or coping with its consequences, but inevitably there will be costs. The same is as true for the disease caused by the Koi Herpes Virus as for any other disease. Thus the ideas presented in this document are not and cannot be free of cost in either time or finance, but nor is the disease.

KEY POINTS IN THE BIOLOGY OF THE KOI HERPES VIRUS

Very little is known in detail about this virus.

KHV is very infectious.

It appears only to infect carp (Cyprinus carpio).

Goldfish and other fish of the carp family are not known to be affected by or carry the virus.

The virus does not appear to survive transport with goldfish.

It may reside as a latent infection within a fish without causing disease for a considerable period.

Outright disease mainly occurs between 23 and 28°C, but it may occur between 18 and 30°C.

No vaccine is available.

The virus appears to have an incubation period of 14 days following the introduction of uninfected fish to infected ones. There are some indications that this period may be longer - this gives rise to speculation that while temperature is the primary trigger a secondary one may be necessary.
HOW CAN WE IDENTIFY THE DISEASE

ON SIGHT

KHV’S CALLING CARDS

KHV should be considered as a potential culprit (not the culprit) if:

· Only carp (Cyprinus carpio) including koi, ghosts and common varieties are affected and killed. If other species such as goldfish and grass carp in the same system remain unaffected even if diseased carp are present, then the presence of KHV must be regarded as a candidate for being the culprit.

· Mortalities occur at water temperatures between 18-30°C. If during an outbreak the temperature rises above or falls below this range mortalities diminish or stop.

· Mortalities are very rapid. Seemingly healthy fish become ill and die in 24 to 48 hours.

· Very severe mortalities. 80-100% mortalities occur within 10 days of disease outbreak.

CLINICAL AND PHYSICAL SIGNS OF KHV

· Bleeding from the gills (that may also release virus and part account for its’ infectious nature).

· White patches, (where excess mucus has been produced or tissue has died) which may be small to very extensive, on the gills.

· Pale patches on and blistering of the skin.

· Sunken eyes.

· Occasionally fish have shown signs of nervous problems (that is periods of inactivity followed by hyperactive behaviour triggered by a very small stimulant). This sort of activity could be due to suffocation as the gills become more and more clogged with mucus through which the absorption of oxygen becomes more difficult.

The Koi Herpes Virus seems to suppress or inhibit the fishes immune system. The body can’t then react as it would normally to prevent infections. Thus secondary invasions of bacteria and parasites might be the most obvious problem on an individual fish, but these signs may obscure the damage caused by the virus.
UNDER THE MICROSCOPE

Damage to the gills may be seen by using wet preparations. You may also just be aware of large numbers of bacteria and parasites in the mucus. This is the limit of the investigations possible in even those retail outlets possessing and using a light microscope, and a diagnosis cannot be made on this information alone.

In heavily infected fish, scientific laboratories are able to prepare and stain very thin sections of tissue (this is known as histology) in which they may be able to see signs characteristic of KHV. Under extremely powerful electron microscopes individual virus particles can be seen.

POLYMERASE CHAIN REACTION - PCR

WHAT IS PCR?

PCR is a technique used to produce a large number of copies of a specific region or sequence of an organism’s genetic code, the DNA. The DNA code or sequence is unique to the organism of interest, and by amplifying this specific sequence using the PCR a very small amount of DNA can be amplified to amounts that can be detected.

The method becomes even more sensitive as scientists are able to increase the number of the segments they can detect in a sample making detection easier. If not one, but two sequences of unique genetic code are used for detection. These “nested” PCR tests may be more accurate. It has been said that this technique could detect a single viral particle in a sample.

SO WHERE’S THE PROBLEM - IN GENERAL TERMS?

The method doesn’t necessarily detect the whole disease organism.

PCR’s are described as proxy tests, that is they detect the presence of small chains of genetic material not necessarily a whole organism. Thus a PCR can detect the debris of organisms long since gone, or certainly no longer capable of producing disease. As herpes viruses enter lengthy latent periods this may be a benefit rather than a problem, as a PCR test could potentially pick up the viral debris from a prior infection.

False results

The standard method can show false positives and negatives if the methods are not very carefully controlled in other diseases. Vaccination might cause false positives.

The clinical signs associated with KHV disease can be very similar to those of many common bacterial infections and parasite infestations. The presence of KHV cannot be definitively determined purely by observing the fishes appearance or clinical signs alone.
The consequences of false negatives
Missed opportunities to manage a disease outbreak or spread which could lead to economic harm.

The consequences of false positives
Missed opportunities to import or export. Stock slaughtered unnecessarily.

So where’s the problem - specifically with KHV in mind?
The KHV PCR is not yet as well developed and accurate as is desirable. Though a more accurate nested PCR for this virus is in the later stages of development, it is not available now.

The KHV PCR that is available is relatively insensitive and it will only detect the presence of the virus when fish are showing very well developed clinical signs. It will not detect latent virus. Thus it is much more likely to give a false negative than a false positive. This fact must be very clearly understood if the current PCR is used in any form of screening program.

As the state of knowledge stands at the moment, a negative PCR result must not be taken as giving any guarantees or assurances beyond the fact that the virus could not be found in a batch of fish at a particular time. This may mean little more than the fish were not ill at the time, that is, there were no well developed clinical signs. No absolute claims to be “disease free” or have “virus free” status can or should be made on the basis of these tests.

On the other hand a positive test is a pretty definitive statement that present in those fish at that time was a high population of the Koi Herpes Virus.

Undertaking screening tests may help satisfy your customers (and possibly the courts should the need arise) that you have taken all reasonable care to avoid supplying fish infected with KHV.

At least 16 cases in the UK, 45 cases in Germany (found by a German lab), one case in Holland and 2 cases in the USA have been confirmed using PCR technology over the last year. We must presume any case reported in Israel has also used this technology.

The PCR test available may be used to help screen sources of supply. Care should be taken to understand when this test may be used and how any resultants may be interpreted.

Cost of the KHV PCR
The Fish Disease Laboratory (CEFAS) at Weymouth have indicated the approximate costs of PCR tests for screening a maximum of 12 pools of tissue will not exceed £400. (This includes costs, consumables and chemical reagents).

The 12 pools will consist of:

- 6 x gill + brain tissue pools
- 6x liver + kidney + spleen tissue pools
A pool will consist of tissue excised from 1 to 5 fish, ie a minimum sample of 6 and a maximum sample of 30 fish.

So, screening 30 fish as pools of 5 = £400 but screening 30 individual fish would cost £400 x 5 = £2000.

The cost of the tests also includes sampling of the fish carried out by CEFAS staff (at no additional charge).

Members must confirm costs and the methods to be used to be most effective with: Dr. Keith Way at CEFAS, telephone 01305 206600.

In other countries state veterinary laboratories may offer the facilities to undertake this PCR. Dr Ron Hedrick at UCL Davis and his students are also working on KHV.

Remember, for the time being the PCR test can only detect virus in fish showing clear signs of the disease. When testing for the presence of some diseases, taking a certain number of samples means the presence of a disease, in say 2% of the population, can be established with a certain level of confidence. Using the current PCR on seemingly healthy fish will not give you useful information about the presence or absence of the virus.

**ELISA***

If any of you have been unfortunate enough to be involved in an investigation of a possible SVC outbreak, you may well have seen samples for ELISA testing being taken. These tests very rapidly give an indication of the presence or absence of a disease. The results are only presumptive and require confirmation by a cell line isolation test before confirmation. Samples from clearly diseased fish are returned to the laboratory for this test.

They rely on a level of knowledge of the antibodies produced by the fishes immune system to a particular pathogen which is not yet available for KHV. Latent infections may not produce very large amounts of antibodies making the situation even more difficult. It is unlikely that an ELISA test, even if available would be any better than the PCR.

*Enzyme Linked Immunosorbent Assay

**CELL LINE ISOLATION**

Cells can be grown in thin layers in shallow dishes. A whole range of types of cell are now grown in labs routinely, for instance Fat Head Minnow (FHM) and Koi Fin (KF) cells.

Some viruses such as the SVC virus grow in a wide range of cell lines, but others can be very specific and only grow in cells derived from the original host. KHV can only be grown in koi fin cells.

Viruses can be inoculated on to these cells. Not all viruses will infect all cells. Cells that become infected will show definite signs of infections, these signs may be specific to a particular virus. The PCR can be used as a confirmatory tool, though it is not entirely suitable for this job in the case of KHV.
CAN WE PROTECT OURSELVES?

SELECTION OF SOURCE OF FISH

If the source of your fish hasn’t been infected with KHV then the fish you buy can not carry the virus. The following criteria and questions might be helpful in formulating enquiries to suppliers:

- Has the site ever been tested for KHV? If so when and what were the results? By whom were the samples selected and collected? Which laboratory undertook the tests using which method - PCR, microscopy (technically known as histopathology) etc.?

- Has the site a protected water source (that is one in which there are no fish) such as a well or a borehole? Are barriers in place to prevent fish swimming upstream into the site? What precautions are in place to prevent birds entering the site or dropping live fish into pools on the site? This will help ensure that infection cannot occur from local native fish stocks.

- Have the fish been mixed with stock from any other source in the supply chain? KHV is very infectious; any mixing of fish from different sources risks cross infection. This is a particularly pertinent question to ask of exporters or importers who consolidate stock from either a wide geographic area or a number of production units. The more stages there are in the supply chain, the more potential for cross contamination if mixing occurs.

- Have any fish been brought onto the site recently? If so from where, and what precautions were taken at those sites to prevent the entry of problems?

The OATA survey of fish health 2001 revealed that a number of countries including China, Israel, Japan, Malaysia, Thailand and the USA were mentioned as sources of stock affected by these disease outbreaks. The difficulty of diagnosing this disease, means these findings cannot necessarily be regarded as absolute proof that KHV was present in fish sent from those countries. No such conclusion should be drawn from this paragraph. Indeed it may mean the fish concerned were naive and infected by cross contamination from other fish on site.

Further suggestions may be found in the DEFRA (formerly MAFF) document advice to avoid SVC included at Annex 2.

Very careful questioning of sources of fish supplied using the type of questions outlined in this report should be a first step to managing the risks presented by KHV. This might equally apply to importers (both wholesalers and consolidators) seeking assurances from exporters, as to retailers seeking assurances from wholesalers or consolidators.

CAN WE QUARANTINE FISH (AGAINST KHV OR ANY OTHER “DISEASE”)?

The simple answer is generally NO but may be YES.
The term quarantine is defined as “isolation imposed on persons or animals that have arrived from elsewhere or been exposed to, and might spread, infectious or contagious disease.” It is derived from the Italian *quarantina* which means forty days. Quarantine (that is a forty day period of isolation) can be applied to any animal, but was originally applied to humans and warm blooded animals. The expectation was that forty days (or other period of time stipulated by law) would be longer than the incubation of serious diseases like small pox or rabies. Thus any infected animal would become identifiably unwell in that period.

Temperature is particularly important in the development of KHV.

Warm-blooded animals have a metabolism that keeps their body temperatures stable within a narrow range. Disease causing agents of these animals have adapted to be infective within that narrow temperature range. This is why the incubation period, the time between exposure to infection and signs of disease becoming evident, of diseases in warm blooded animals can be predicted with some accuracy.

Fish are not warm blooded, and their diseases do not have incubation periods that are similar in all conditions. Fish adopt the temperature of the water that surrounds them. If their environment is temperature stable, then the incubation period of a disease may be predictable. However most fish are subject to quite wide fluctuations in temperature, koi can be in found in water close to freezing or up to temperatures of 30°C or more, and there are wide differences in incubation periods for diseases across this temperature range.

However to a larger degree than in many other animals, stress predisposes fish to disease. Stress does not cause these diseases as the pathogen must be present. Unless the trigger, frequently stress, is present a disease will not develop even if the pathogen is present.

**ACCLIMATISATION**

An acclimatisation may be a more accurate description of what is usually described as “quarantine”. Basically the fish are rested and kept in optimal conditions which are as stress free as possible. It could be argued that these are the conditions least likely to precipitate full-blown disease in fish. Thus acclimatisation may achieve the complete opposite of quarantine as used for mammals.

**PREVENTATIVE ACCLIMATISATION**

However an acclimatisation period may be used to try to determine if the fish are carrying KHV.

Each batch of fish must be isolated, this is a normal requirement of any good husbandry or stock system. Clearly there are costs associated with meeting this requirement, but these may prove to be less than those incurred during outbreaks of this disease at your establishment and/or that of your customers. No transfer of water between batches must be permitted including that on hands, nets, aerosols (eg the droplets formed by working airstones), wellingtons or buckets.

The fish are then kept at between 23 and 28°C for at least two weeks. A sample of fish showing clinical signs of disease would then be tested for the virus at the end of that period.

Each batch of fish should be isolated completely. No transfer of fish, water or equipment should be permitted. If you require exporters to give assurances as part of the contract of supply, they
may require that you provide evidence of an effective isolation policy of new stocks, and its effective practical application. Basic records of water quality, mortalities and observations of fish stocks should be maintained and held for reference.

Fish should be subject to a period of “preventative acclimatisation/isolation” of at least 14 days, ideally longer at 23 to 28°C. Any batch of fish in which signs of illness or disease and particularly showing mortalities similar to what this report has called the “calling card” of KHV should be subject to PCR tests. If these prove positive, then that source of supply may be considered unsuitable until adequate remedial action has been undertaken.

Batches from major sources of supply might usefully be screened prior to the start of the next coldwater season. To be confident of the results of such screening, the isolation policy must be absolute. Given the apparent infectious nature of the virus in closed systems no chances of cross contamination should be tolerated if the PCR results are to be useful.

All water should be discharged via the foul sewerage system.

No koi or other carp should be accepted from any unknown source, including retail customers, and returned to your holding systems.

**SIZE AND FREQUENCY OF IMPORT CONSIGNMENTS**

The risk of losses may be reduced by importing smaller consignments more frequently than perhaps has been the custom in the past. However this may require more investment in isolation facilities.

**CAN VIRUSES BE TREATED?**

Generally no. But…. Treatments for cold sores in humans are available which are rubbed on the sores. However these just deal with the virus present close to the skin not with any in nerve tissue or other internal organs. Other treatments are claimed to diffuse down nerve cells and thus treat the virus. Articles have appeared on the web that advocate the use of these treatments for KHV. Even if these treatments of the human cold sore herpes virus were active against KHV it must be remembered these drugs are licensed for use only in humans. Additionally the damage and stressed caused to the fish by repeated handling to rub cream in may not be a very good option. Finally it is unlikely that a treatment intended for use on human skin will work when applied to a wet fish, and immediately immersed in water.

**IMMUNISATION**

**VACCINES**

The most usual way of immunising animals is by vaccination, as humans are against diseases such as polio. Vaccines have been developed for some herpes viruses eg that infecting turkeys. None are currently available for KHV.
IMMUNISATION BY EXPOSURE TO DISEASED FISH

Various methods of using exposure to diseased fish to "immunise" koi against KHV have been proposed. These generally rely on exposing to fish to infected fish between 20 and 28°C (when the virus will be at its most active) for a period, and then raising or lowering the temperature so that the virus is partially inactivated and the bodies immune system is able to kill it. By doing this it is hoped the body will be immunised for life.

NATURALLY IMMUNE FISH

Some strains of a species may be more resistant, or even immune, to an infectious agent than others. Thus it is possible that strains of carp naturally immune to KHV have or will emerge. Also in some instances viruses may become less virulent with the passage of time, and thus though present may not cause disease. In both instances they may be capable of acting as carriers and thus acting as sources of the disease and possibly spreading it if transported.

Any claims to supply immunised fish or fish which are naturally immune should be accompanied by written assurances as part of an enforceable contract of supply that these fish are not carriers of latent KHV infections. Once again in the event of a claim, you may be justifiably called upon to establish the efficiency of your isolation policy.

USE OF ANTIBIOTICS

As stated above KHV is apparently not infrequently accompanied by a massive growth in bacterial and parasite populations. There is some evidence that if these secondary invaders are treated then the fish may stand a greater chance of survival. However, even if the widespread use of say antibiotics were feasible, it would not necessarily solve the problem and may cause new ones. Even if all the secondary bacteria were killed and the fish survived, the fish may still carry latent KHV.

Unsupervised use of antibiotics would almost certainly lead to resistant bacterial strains that might themselves cause disease, and would then be difficult or impossible to treat.

Indiscriminate use of antibiotics kills a wide range of harmless bacteria, not just those causing a disease. These harmless bacteria occupy body surfaces, and their presence makes that surface unavailable to disease causing bacteria. Thus in practice mis-use of antibiotics may make diseases more likely to occur.

Properly supervised administration of correctly chosen antibiotics may enable some fish to survive outbreaks. However these may remain carriers of KHV.

MANAGEMENT OF FISH SURVIVING OUTBREAKS OF KHV

As noted above KHV causes very high rates of mortality. However there are survivors. It could be argued these are naturally immune or immunized fish and hence very useful. On the other hand they could carry latent virus.

Fish surviving outbreaks of KHV should be regarded as potential carriers of the disease. It may prove unwise to mix these fish with any other stocks. Members of the public presented with this
situation may choose to dispose of the remaining stock and buy new fish, or maintain the lower stocking. Even if survivors are genetically immune, this trait may not be passed on to the next generation.

**DISINFECTION**

There is no evidence that KHV can survive in the environment without a host. It seems pretty certain that KHV hasn’t been carried with goldfish. Also no further problems were apparently encountered in a system into which no carp were put for three months. However to rely on the technique of removing susceptible species for a lengthy period alone may be both unwise and commercially impractical.

A more certain method is to completely strip any system in which infected fish have been. The filters must be disinfected, as it is not beyond the bounds of possibility that the bacterial films and other organic matter could allow reservoirs of infective virus to persist.

Disinfection, following a suspected outbreak of KHV or indeed any serious disease, may be carried out using the techniques outlined in Annex B by DEFRA (formerly MAFF) for SVC. Further advice on this technique or alternatives is available from the Fish Health Inspectorate at CEFAS T: 01305 206673/4 or fishhealthinspectorate@cefas.co.uk
THE FUTURE

COULD KHV BE MADE A NOTIFIABLE DISEASE?

Notifiable diseases are those to which countries may most easily produce import rules with a view to preventing their entry. If rules are put in place then they need to avoid obstructing free trade. The rules on what constitutes a barrier to trade is determined by the World Trade Organisation.

If KHV were made notifiable in the UK, a two year ban (at least) on carp imports (including koi) would follow.

THE WORLD TRADE ORGANISATION (WTO) RULES

The World Trade Organisation rules allow each country the right to protect human, animal and plant life. This could mean the implementation of import rules, but these must be:

- based on science;
- be justified with scientific evidence;
- not be disguised barriers to trade;
- only applied to the extent necessary to give the level of protection required. That is, the import measures must be commensurate with the risk.

Exporting countries have the burden of proof that their live materials or products are safe. However the controls and tests they apply need only give equivalent protection to those used in the importing country. They do not need to be identical. Once again the burden of proving equivalence would rest with the importing country.

The WTO recognises the OIE (a body concerned with international animal health matters based in Paris) as a “standard setting organisation”. Thus if import rules and regulations are based on their guidelines they are likely to meet the WTO criteria. Restrictions on trade must be the least trade restrictive possible to meet the goal of preventing the entry of a particular disease. The “zero risk” philosophy in which any animal or fish movement is seen to pose some risk, which would quickly end any international trade in any animals, is no longer acceptable. This requires that importing and exporting countries trust the competence of one another’s veterinary service.

THE SITUATION AS IT APPLIES TO KHV

WTO rules permit a country to protect itself, by implementing imports rules, from specified diseases. There is a presumption that it is known where the disease is present and that it is not present in the country applying the import rules. As there is as yet no test for KHV that is accurate at all states of the disease, and it is impossible to know the exact geographic range of the virus. Unless an accurate test is available a country may establish barriers against a disease that is widespread within its borders but doesn’t know it. For instance the virus might be present in cooler countries but never cause disease. This uncertainty means there may be reluctance by any country to make this disease truly notifiable as understood in EU or the OIE rules.
ANNEX A

OATA ACTION PLAN ON THE KOI HERPES VIRUS (KHV) – SEPTEMBER 2001

The following action plan was formulated by directors following a meeting of members held between 2.30 and 5.30pm on Wednesday 5th September. Nick Grierson of the Pet Care Trust and Adrian Barnes of the Professional Koi Dealers Association were present. It is intended that this work will help members cope with the inherent uncertainties that the current lack of knowledge causes, particularly in view of the proposed changes in consumer law.

i) The key aim of this plan is to ensure that confidence is maintained or restored in koi in the public domain.

ii) OATA should seek to co-operate with other trade groups in identifying the current range of KHV, and formulating a response by industry to manage or eradicate the problem.

iii) As knowledge of the virus develops any plan should adapt to and adopt current best information.

iv) From as many legitimate sources as possible, a database of “incidents” that may be related to the presence of KHV should be organised. This should be kept in the OATA office. No results should be divulged that could identify reports from individual businesses should be prepared. Consolidated reports which will prove useful to the understanding of the disease and its spread should be prepared, and used as appropriate at a particular time.

v) From this database, areas or sites that are suspected to be sources of the virus (hot spots) and those presumed clear (cold spots) of the virus may be prepared.

vi) DEFRA/CEFAS should be approached to:

• Determine the practicality and expense of using the current PCR, cell isolation or other tests in determining the distribution globally and locally of the virus. Priority may be given to determining the validity of using the database in 4 above to determine the presence or absence of the virus from a source or country.

• Ask that funding either LINK Aquaculture style or from central funds be used to study the biology of virus to improve our understanding of it and our management (or eradication) of it with time.

• Identify and exploit any other opportunities for co-operation that will help to more speedily resolve this problem.

vii) If and when legally appropriate, publicly announce the results of the work concerning “hot spot” and “cold spot” sources of supply.

viii) Prepare an information sheet for members on the virus.

ix) Prepare a best practice guide for members including issues, of detection, acclimatisation (quarantine), isolation, disinfection and so forth.
ANNEX B

ADVICE TO DEALERS, TRADERS AND FARMERS ON STEPS TO PREVENT THE SPREAD OF SVC

Taken from: ‘Spring Viraemia of Carp’, produced by Ministry of Agriculture, Fisheries and Food Welsh Office Agriculture Department (now DEFRA) several years ago. Copies can be obtained from MAFF Fish Diseases Laboratory T: 01305 206600.

Dealers, traders and farmers should report any knowledge or suspicions of SVC to the Fish Diseases Laboratory, Weymouth or to their local Water Authority.

Carp, just like any other fish, have increased susceptibility to disease when they are stressed. Stress can be caused by a number of factors such as rapid changes in water temperature, movement from one site to another, poor handling or overstocking. The risk of disease and mortalities can be significantly reduced by keeping stress to a minimum wherever possible.

Dealers should avoid collecting and mixing fish from different sites, especially when the fish are intended for immediate sale. If feasible, dealers should also consider a period of quarantine (preferably for two weeks) for recently acquired stocks. This may prevent the introduction of SVC to their own stocks and to those of customers. When netting stocks, dealers should ensure that nets and equipment are disinfected before use at another site.

It will obviously benefit dealers, traders and farmers to ask appropriate questions of importing agents or wholesale suppliers as to the health of fish before accepting consignments.

Importers of ornamental varieties should take all reasonable steps to determine whether overseas suppliers can give assurances that their fish stocks are free from infection with SVC virus, or other serious diseases. Importers should consider seeking satisfactory responses from suppliers on points such as:

- Are regular examinations for disease carried out on fish stocks held on the supplier’s site/premises?
- Are all new consignments of fish brought into the supplier’s site/premises examined for disease?
- Who undertakes these examinations (e.g. own employee, private veterinarians, state veterinary service)?
- Are only sick fish examined, or are random samples of apparently healthy fish also examined routinely?
- Are samples taken for laboratory tests for bacteria, viruses, parasites? If yes, which laboratory carries out the test?
- Can the supplier provide a list of tests carried out on his site/premises and the results?
- What steps does the supplier take to ensure that the health status of his own supplier’s fish stocks is acceptable?
DISINFECTION

Ideally, disinfection should involve the treatment of nets, sacks and footwear and other equipment such as boats, prior to moving to other waters. Equipment used in fishery remedial work, such as bank repairs and stock netting, might similarly be treated.

Iodine-based preparations (iodophors) are recommended for disinfecting equipment: such preparations, by way of example, include Wescodyne (Ciba Geigy Ltd) and FAM-30 (Vanodine International). Advice on local sources of supply or suitable alternatives may be obtained from local veterinary surgeons, Water Authorities, or the Ministry’s Fish Disease Laboratory at Weymouth (T: 01305 206600). As a broad guide Wescodyne should be diluted: 1 ½ parts in 100 with water and FAM-30: 1 part in 100 with water. Other iodophores should be diluted to provide a final concentration of 250 parts per million active iodine. Suppliers, or scientists at the Fish Disease Laboratory, Weymouth can advise in cases of doubt. Disinfection is best achieved by first cleaning off all mud etc followed by immersion for 15 to 30 minutes; or by application to surfaces using a pad soaked with disinfectant. (Reference to specific disinfectant products should not be construed as any criticism of similar products).

For regular disinfection of heavily soiled footwear, a bath of 1% caustic soda (sodium hydroxide) solution is more appropriate. The strength of the caustic soda should be checked daily and the disinfectant replaced if the pH is 11 or below, as shown by indicator papers. Protective clothing should be used when handling caustic soda to protect the skin and eyes. This disinfectant can corrode metals.

Iodophors and many other disinfectants are extremely poisonous to fish. Footwear and all equipment, especially nets, should be thoroughly rinsed with tap water after disinfecting. Disinfectant and washings must be disposed of in a way which does not harm the environment. They should never be tipped into water containing fish or other aquatic life.
ANNEX C

DISPOSAL OF CLINICAL WASTE

Two years ago in a BBC “Watchdog” program about the company “Petsmart” concerning their care of mammals, the issue of the disposal of dead animals was raised. The program alleged that dead animals that should have been treated as clinical waste, were thrown in the rubbish skip rather than in “yellow bags”.

WHAT IS CLINICAL WASTE?
“Clinical waste” includes any waste which consists wholly or partly of human or animal tissue, blood, other body fluids, excretions, dressings, syringes or needles which unless rendered safe may prove hazardous to any person coming into contact with it. Dead fish would appear to fit into this definition. Certainly the bodies of dead animals from veterinary surgeries are defined as clinical waste.

A fuller definition is given in the Controlled Waste Regulations 1992.

RISK ASSESSMENT
As with any operation in the work place, a risk assessment may be required for operations handling dead animals under the Health and Safety or COSHH legislation. This should include any risks to health from handling dead animals, including any precautions thought necessary (in the case of fish good personal hygiene and the provision of gloves may reduce any risks from zoonoses).

HOW SHOULD CLINICAL WASTE BE DISPOSED OF?
In yellow plastic sacks provided by a specialist collection company. They can then be picked up at appropriate intervals.

DO I HAVE TO TREAT DEAD FISH AS CLINICAL WASTE?
Dead fish may come within the definitions in the relevant act. However if you wish to treat dead fish as ordinary waste, you would have to prove to the relevant authority that it constituted no greater risk than ordinary waste. Advice on appropriate treatments may be available from the EA, SEPA or HSWE.

Already one member has reported discussing this issue as part of his pet shop licence renewal. The conclusion reached was that so little waste was generated, that it could be treated as ordinary waste material.

As you may be aware, each district authority can interpret the law differently. Thus before incurring any costs it would be wise to seek their advice on what they deem appropriate.

FURTHER READING AND INFORMATION
Safe disposal of clinical waste – Health Services Advisory Committee published by HSE books, PO Box 1999, Sudbury, Suffolk CO10 6FS Tel: 01787 881165 Fax: 01787 313995.

The Health and Safety Executive also provide the HSE Infoline T: 0541 545500
Or you may write to HSE Information Centre, Broad Lane, Sheffield S3 7HQ.

or
www.open.gov.uk/hse/hsehome.htm

Further advice may be available from your local Environment Agency office in England and Wales and the Scottish Environment Protection Agency (SEPA).
ANNEX D

FISH EUTHANASIA

It now is widely accepted that fish feel pain or something similar to it. Euthanasia must be carried out using a technique that brings about death quickly with as little pain as possible. Fish farmers and anglers routinely use a well aimed blow to the head which kills fish rapidly.

If faced with a large number of fish to destroy, a member may be best advised to seek help from his or her vet. A fish anaesthetic such as benzocaine which is highly effective may be considered. Fish are netted into a tub of benzocaine where they are allowed to become overdosed.

Benzocaine is relatively insoluble in water. It must first be dissolved in alcohol or acetone at a rate of 40g to the litre.

This is then dissolved in water at a rate of 20ml per 2 gallons (9 litres) which gives a benzocaine level of 100 ppm. This will rapidly anaesthetise the fish. Fish should be left in the solution for 2 hours to ensure that death has taken place.

Dead fish should be disposed of with due consideration to health and safety, and in accordance with disposal of waste legislation that may be applicable. They should not be thrown in the bin!