

The distribution of Koi Herpesvirus (KHV) in England and Wales

2007 to 2008 Report

Nick Taylor, Keith Way, Peter Dixon, Edmund Peeler, Keith Jeffery
& Kevin Denham.

Cefas, Weymouth Laboratory, Barrack Road, Dorset, UK. DT4 8UB.

Summary

Koi herpesvirus (KHV) causes a highly virulent disease affecting carp (*Cyprinus carpio*) and poses a serious socio-economic threat to the UK carp industry. This study aimed to determine the geographic distribution and prevalence of KHV in England and Wales in order to determine the practicality of different statutory control options under consideration by Defra. Carp were tested for the presence of KHV antibodies in their blood using an Elisa. The study examined carp from 82 farm sites, 72 fisheries and 12 consignments of ornamental carp imported from seven different S.E. Asian countries.

Three of the farms sampled produced positive results. These results suggest that UK fish farms are a relatively safe source from which to obtain fish. Fish farms do, however, have the potential to spread the virus rapidly due to the number of contacts they make. Consequently it is of great importance that infected sites are detected early and steps taken to prevent spread.

As the primary aim of the study was to establish the geographic distribution of KHV exposed fish, those fisheries deemed to be at highest risk of exposure were sampled. These were sites receiving high numbers of consignments of fish, or suffering mortalities. Of the 72 fisheries tested, 26 were positive. The results indicate that none of the geographic areas studied were KHV free.

Consignments of imported fish from six countries tested positive for KHV antibody. Although a high proportion of consignments were positive the results indicate that lower risk stocks of fish exist that could be sourced by the industry.

Although widespread and prevalent in 'high-risk' fisheries, there are good prospects for KHV control as our farms appear to relatively free of the virus,

and there are low risk stocks of fish from third countries that could be sourced. Additionally similar controls to those in place for SVC may be effective at controlling KHV in fisheries.

Introduction

Koi herpesvirus (KHV) causes a highly virulent disease affecting carp (*Cyprinus carpio*) and poses a serious socio-economic threat to the UK carp industry. From analysis of archive histological material, Cefas has putative evidence for the presence of KHV in England since 1996 when viral DNA was detected in a warm water re-circulation facility in Derbyshire (Haenen *et al.* 2004). However, until 2003, KHV had only been detected and isolated from sites in the UK holding imported ornamental carp from Israel, Malaysia, USA and Japan. In 2003 Cefas detected KHV in common carp during investigations into large mortalities in angling waters (Denham 2003, Way & Dixon 2007). Further detections of KHV were subsequently made at a small number of angling waters in 2004 and 2005. In 2006 KHV disease outbreaks were reported and confirmed at 23 sites in southern England (Way & Dixon 2007).

KHV disease has been made an OIE listed disease and became a notifiable disease in England and Wales in April 2007. KHV is also listed under Council Directive 2006/88/EC, thus subjecting the disease to community controls. All EU member states will have to submit their health status with regard to KHV by 1st August 2008. As a result the geographic distribution and prevalence of KHV in England and Wales needs to be assessed in order to determine the UK's status and the practicality of different statutory control options under consideration by Defra. This study aims to assess:

1. The types of fish that have been exposed to KHV – ornamental, fishery and farmed.
2. The geographic distribution and prevalence of the infection within each sector (identified in 1. above).

Materials & Methods

Target sample size for each site or fish consignment sampled was 30, as this provides a 95% confidence of detecting exposed fish if they constitute $\geq 10\%$ of the population from which they originate (smaller sample sizes would decrease the level of confidence in detecting and exposed fish at this prevalence). This level of sampling confidence was deemed most appropriate given the timescale and resources available to the project. Fish sampled were euthanised using an appropriate method and blood samples taken. Where this was not possible due to the value of the fish, they were anaesthetised, blood was taken and the fish were allowed to recover before release. Blood samples were then chilled and returned to the Cefas Weymouth laboratory where they were centrifuged and the sera removed for later analysis. Sera were screened using the Elisa method developed at the Cefas Weymouth laboratory (St-Hilaire et al. 2005) to detect antibodies to KHV. A site was defined as positive for KHV antibody if one or more fish in the sample produced an optical density of ≥ 0.15 @ 450nm.

To establish the risks posed by imported ornamental carp, consignments of koi carp were obtained from UK based fish importers. Each consignment ordered was from a different non-EU country sourced from several different suppliers. Each consignment contained 30 fish of greater than 15cm in length.

Carp produced at registered fish farms in England and Wales were sampled during the Fish Health Inspectorate's (FHI) routine monitoring programme for Spring viraemia of Carp (SVC). Before taking these samples the project and its purpose was explained to the farm owners and their permission to test for KHV antibody sought. Sites where permission could not be obtained were not sampled. In addition, farm samples collected for the purpose of other studies but suitable for analysis for KHV antibody were held as archived material at Cefas. Sites for which such material was stored were contacted and their permission sought to test for KHV antibody.

Fisheries samples were obtained through two routes. The Environment Agency (EA) were asked to provide blood samples from any carp mortality events that they were investigating, or any section 30 movement consents for KHV antibody testing. The second route was through Cefas fish health inspectors visiting fisheries selected from a targeted population for the purpose of this study. The fisheries targeted were those receiving the greatest number of fish movements in 2006 as these were not only likely to be at the greatest risk of getting the virus but were also likely to be the more intensely stocked waters and therefore the easiest to obtain sufficient fish numbers. Sampling sites were selected by searching the live fish movements database (LFMD) for consented carp movements onto fisheries. Each site identified was listed by EA geographic region (fig. 1). Sites were then ranked in order of the number of carp consignments they had received (highest first). The first ten in each region were sent a letter and fact sheet explaining the purpose of the project (Annexes 1 & 2). Each site owner was then contacted by telephone and asked whether they would be willing to take part in the study, and for their permission to take the necessary samples. Sites for which permission was obtained were visited by a fish health inspector who sampled rod caught fish upon capture, or fish that had been caught and held in nets prior to the inspector's arrival. At each fishery basic data were collected on the site management, stocking and physical characteristics in order to try and establish risk factors for KHV. These data were entered into a database created in Epi-info (CDC, Atlanta, USA) and analysed using basic statistics and logistic regression. Details of historic clinical cases of KHV in fisheries were also entered in to the database and analysed in a similar way to determine common factors. Such analysis was not conducted on farm sites due to insufficient case sites to allow meaningful results to be obtained.

All sites testing positive for KHV were identified on the LFMD and any contacts on or off the site in the five years prior to testing recorded. The regions in which these contact sites were located along with the site type were identified in order to determine potential spread. All suppliers identified to have supplied fish to positive sites were listed and any supplying more than one positive site was short listed for follow-up sampling in futures studies. In

cases where a positive site had only received fish from one source, the sole supplier was also listed for future follow up.



Figure 1. Environment Agency regions used to select study fisheries.

Results:

A total of 82 farm sites, 71 fisheries and 12 consignments of imported Koi carp from third countries were sampled during this study year. The response from farm sites was extremely positive with only two sites refusing to take part in the study. Farm sites were logistically easy to sample and a 30 fish sample was obtained from 74 of them. Where a 30 fish sample was not obtained, this was due to difficulties in obtaining sufficient blood from a small number of fish. The minimum sample size obtained from farm sites was 23.

The response rate from fisheries was low due to reluctance by many site owners to allow samples to be taken, possibly due to a fear of subsequent

ramifications should the site test positive. Of the fisheries sampled, it proved difficult to obtain the target sample size 30 fish in the majority of sites given the time constraints of the study. Of the farm sites sampled three (4%) gave positive results. Of the fisheries sampled 26 (37%) of 71 tested positive. The distribution of sites testing positive is widespread, and at least one positive site was identified in each of the regions sampled. The geographic distribution of the sites sampled and testing positive is presented in Table 1.

Table 1. The geographic distribution of KHV study sites and sites testing positive for KHV by Elisa in England and Wales.

	Farms Sampled	Positive Farms	Farm Prevalence	Fisheries Sampled	Positive Fisheries	Fishery Prevalence
South West	18	2	11%	8	3	40%
Southern	13	1	8%	8	0	0%
Thames	8	0	0%	10	3	30%
Anglian	17	0	0%	14	4	30%
Midlands	16	0	0%	10	6	60%
Wales	1	0	0%	5	2	40%
North West	2	0	0%	11	7	64%
North East	7	0	0%	5	1	20%
Total	82	3	4%	71	26	37%

Imported fish: The imported fish tested originated from 11 different suppliers and seven countries in South-East Asia. None of the samples originated from Israel, which is known to produce vaccinated fish. Of the consignments tested, six were positive. These originated from six different countries and came through six different suppliers.

Clinical outbreaks: Clinical cases of KHV, all of which occurred in fisheries between 2003 and 2006 were reviewed. Of the 29 case sites reviewed, 19 were known to have either koi carp, ghost carp or goldfish present. All sites

experiencing outbreaks had high (>560kg per hectare) or very high stock densities (>1120kg per hectare) with 17 sites having high and 11 very high. Angling pressures (based on EA officers individual judgement) at these sites were: 13 = moderate, 12 = heavy and 2 = very heavy. Ten of the sites had no record of any consented stocking of fish having taken place in the two years prior to the outbreak. Three suppliers were identified that had supplied two or more of the clinical case sites. One of these suppliers was known to trade in KHV vaccinated fish.

Retests and comparison with other test methods:

Four sites experiencing clinical outbreaks of KHV in 2006 and receiving no stock since that time were revisited in 2007 and tested for the presence of KHV antibody by Elisa. Three of these sites produced positive results with 28 of 30 (93%), 9 of 10 (90%) and 22 of 26 (85%) fish testing positive at the sites. This demonstrates that in most cases an immune response to KHV can be detected for at least one year after an acute clinical outbreak and that the prevalence of exposed fish would be expected to be high. Why one site tested negative is a difficult to explain, it is possible that the infected fish died before infecting other stock and the remaining fish actually remain naïve.

Fish Farms testing Elisa positive 2007:

As summarised in Table 1, a total of three fish farms produced antibody positive results. Two of these sites were located in the South West region, one in the Southern region. A fourth farm site in the Anglian region also tested positive, however this site received vaccinated fish from Israel. No further analysis was conducted on this site as any sites it may have supplied would be expected to test positive by Elisa due to fish responding to the vaccine. From these results tentative prevalence estimates can be made which indicate that 4% (95% Confidence interval \pm 2.1%) of our fish farms have been exposed to the virus. Both sites in the South West region were recorded as holding both ornamental and coarse fish species, however in each case only mirror and common carp were sampled. In both sites, two (6.7%) of the 30 fish tested gave an antibody positive response. Neither site

has any record of receiving imported fish. Both sites received fish from other English fish farms in 2006, with one being supplied by three sites and the other by one. A total of ten movements off site have occurred between the two farms since 2005; nine within the South West region and one to the Midlands region. Seven movements were to online stillwaters (i.e. connected in some way to a river system), two were to fully enclosed stillwaters and one movement was to another farm. The positive farm site in the Southern region produces both coarse fish and ghost carp and is recorded as receiving imports of fish from Germany. Again two of 30 fish tested produced an antibody positive response. This site had received fish from seven sites prior to testing and has supplied 35 different sites with carp since and in the five years prior to testing. Of these sites 19 were online stillwaters and 16 fully enclosed stillwaters. Twenty sites were in the Southern region, four in Thames, three in the Midlands, three in the North West, two in Wales, one in Anglian and one in the South West.

Fisheries testing Elisa positive 2007:

Of the 71 fisheries tested for KHV by Elisa, 26 (37%) were positive. This prevalence estimate of 37% is however biased and likely to be inflated as it includes samples received as part of mortality investigations. By removing these samples from the analysis, leaving a randomly selected population of sites, the proportion of fisheries that hold fish exposed to KHV can be estimated at 31% (95% confidence interval $\pm 8\%$). Within the positive sites the incidence of exposed fish was variable ranging between 5 and 100%, with mean=36%, median = 22.5%. Again these figures are inflated due to the inclusion of samples from mortality events. By removing these and again looking at the randomly selected study population within site prevalence range from 5 to 25% with mean 14.75% and median 14%.

The positive population of sites was comprised of different water types that can be summarised as follows: one (4%) was a river, three (11%) were stillwaters located in a flood plain, eight (31%) were online stillwaters, 14 (54%) were fully enclosed stillwaters. This can be compared to the sites

testing negative, 23 (52%) of which were fully enclosed and 22 (48%) of which were online. Given the relatively small sample size for which fishery details were available, logistic regression analysis had relatively little power and no statistically significant risk factors were identified. Differences can however be observed between case and control sites (Table 2.). These differences warrant further investigation as more data become available.

Table 2. Comparison of fisheries testing positive and negative for KHV antibody in 2007. Antibody +ve = one or more fish in a sample produce a response with optical density ≥ 0.15 @ 450nm by Elisa, antibody -ve = a response lower than this threshold. s.d. =standard deviation.

	KHV antibody +ve	KHV antibody -ve
Fishery type:		
Match lake	56%	14%
Syndicate/club	22%	32%
Specimen	0%	14%
Pleasure	22%	40%
Ghost/ Koi carp present	75%	58%
Other ornamentals present	50%	21%
Stock density:		
Low (112kg/hectare)	0%	23%
Moderate (336kg/hectare)	56%	45%
High (560kg/hectare)	33%	32%
V. High (1120kg/hectare)	11%	0%
Fishing Pressure:		
Light	0%	20%
Moderate	25%	40%
Heavy	75%	40%
V. Heavy	0%	0%
Average lake depth (m)	2.04 (s.d. = 0.95)	2.59 (s.d. = 2.33)
Average lake size (hectares)	1.81 (s.d. = 5.95)	2.03 (s.d. = 8.30)

Few of the fisheries testing positive for KHV antibody have made consented movements of fish off site since 2002. Only four were identified and these have made a total of nine movements between them. Three were to the South

West, three to the North East, three to the Anglian region and one to the Midlands.

Since 2002 the fisheries testing positive for KHV antibody have received 49 consented consignments of carp from 33 different sites. Four of these supplying sites were identified as supplying two or more of the positive sites. Eight of the positive sites had only received consented consignments of fish from one supplier since 2002 and a further four showed no record of receiving fish since 2002.

Discussion

Prior to this study, little was known regarding the distribution of fish exposed to KHV virus in England and Wales or their occurrence within the different industry sectors. Data from this study and knowledge of previous clinical cases of KHV show that fish exposed to the virus are widespread and can be found in all the regions studied. This finding is in keeping with the knowledge that KHV has been present in the UK for at least 12 years with few legal mechanisms for introducing or applying formal controls to prevent its spread. This information would suggest that the designation of geographical areas that are KHV free is likely to be difficult. Under Council Directive 2006/88/EC it may however be possible for individual sites to gain disease free status, following a targeted surveillance programme, if fish are only obtained from approved KHV free sources, there is a protected, safe water supply and biosecurity is sufficient to prevent introduction via people, equipment, animals or birds. In addition, under this Directive, groups of farms sharing a common biosecurity system, which included a protected water supply, may be able join to form a disease free compartment to allow trade in fish between sites, even if these sites are not in the same geographic area. This option is unlikely to be feasible for many coarse fish farms and fisheries.

The results from consignments of imported koi carp show that fish exposed to KHV are likely to be common amongst those imported and therefore pose a risk of introducing the virus to the UK. It is encouraging that not all

consignments tested positive, showing that stocks posing a lower risk could be sourced by the industry.

Approximately half the fish farms in England and Wales recorded as holding carp have been tested for KHV antibody. At each of the farm sites tested, a good sample size was achieved and we can therefore have a high degree of confidence in the results obtained. Very few farm sites were shown to be positive for KHV antibody and no sites showed a high prevalence of positive fish. This suggests that English and Welsh farm sites have not been experiencing clinical outbreaks and are likely to be a relatively safe source of pathogen free fish, however those fish testing antibody positive have the potential to be carriers of the virus and could therefore be of high risk. It is important that any potentially positive farm sites are identified as soon as possible as they are likely to spread the virus widely and rapidly if infected. Of particular importance is that farm sites identified as having contact with infected fisheries are followed up urgently in order to rule them out as being responsible for the spread of the virus. The three farm sites identified as antibody positive as part of this study made 45 movements to other sites since 2002, thus highlighting the potential for spread should a farm be infected. Many of these movements were to online fisheries, which pose a theoretical risk of transmitting the virus to the wild. To the best of our knowledge the fish tested at these antibody positive farms were not vaccinated. It is not clear why KHV exposed fish were found at the positive farm sites; however, all three sites were mixed producers producing both coarse and ornamental strains of carp. As already discussed, a high proportion of ornamental carp imported to the UK from third countries had been exposed to the virus and are a likely route of virus introduction. As a result, mixed ornamental sites are at relatively high risk of not only becoming infected with, but also spreading the virus if they receive fish that have been imported or been exposed to imported fish. Such mixed farm sites currently comprise 50% of our registered cyprinid farm sites, however the majority are not importing fish.

Although compared to the total population of fisheries present in England and Wales the number sampled was relatively small, it is clear that a substantial proportion carry exposed fish in substantial numbers. The total number of carp fisheries in the England and Wales is unknown, however from the LFMD we can tell that carp movements have been consented to 5192 fisheries from 2227 different waters since 2001 (Table 3).

Table 3. Section 30 fish movement consents granted to fisheries for all strains of Carp (*Cyprinus carpio*) between the start of 2001 and end of 2007.

Year	Supplying waters	Receiving waters	Total movements
2007	447	1215	2023
2006	489	1299	2278
2005	489	1231	2127
2004	526	1244	2782
2003	491	1005	2657
2002	434	855	1740
2001	371	685	1053
Total	-	-	14660

Although high-risk sites receiving high numbers of fish were targeted in this study and few low stock density specimen waters could be tested, it should be remembered that at many of the study sites only small numbers of fish could be sampled, thus reducing the likelihood of detection (sites with a low prevalence of KHV antibody positive fish are most likely not to have been detected when only a small sample size was obtained). The prevalence estimates obtained from the sampled fisheries could therefore be higher than estimated. Movements of fish between fishery sites are likely to be of far greater risk than movements from farms to fisheries. Table 3 shows that since 2001 (the earliest date when reliable fish movement records are available) around 500 sites supply carp to around 1200 fishery sites each year, and in total approximately 2500 movements of carp are consented annually. However, overall few consented movements of fish off site had been made by the fisheries testing positive in this study, suggesting that fisheries play a less significant role in the direct spread of the disease than infected farms. Many of the fish movements that occur between fisheries

involve species not known to be susceptible to KHV, thus, further reducing the risk of spread. The risk of indirect spread via virus contaminated water does however appear to be real, with 31% of fisheries testing positive for KHV antibody being online and therefore connected to river systems. KHV antibody positive fish were detected in a river site as part of this study. This demonstrates that exposed fish may be present in some wild systems either as a result of direct stocking, fish escaping from fisheries during flooding, or through exposure to virus released from online stillwaters.

Many of the fisheries testing antibody positive are not known to have experienced clinical outbreaks of the disease. This may simply be due to lack of reporting, or that the disease has expressed in a chronic as opposed to acute state causing background level losses that have not been investigated. It is also possible that KHV requires certain environmental conditions in order to allow disease expression. Analysis of data from clinical outbreaks shows that these sites tend to be heavily stocked and fished and it is therefore possible that stress plays an important role in disease expression. It should however be remembered that to date no clinical cases have been reported in farms, which are likely to be run at far higher densities than most fisheries. Stock density could be a spurious factor that confounds with high frequency of stocking, which would be expected to increase the risk of exposure to the virus and therefore an outbreak. Equally it is possible that stock density is a risk factor, but only significant when interacting with other risk factors that may not be present on fish farms. The role of temperature in relation to the timing of introduction of the virus may also be important. If introduced when temperatures are not conducive to disease expression, it is possible that a sufficient proportion of the population could have developed sufficient immunity to prevent a clinical outbreak or eliminate the virus by the time the temperatures are conducive to disease expression. This is an area of work that warrants further investigation, possibly through a modelling approach. Alternatively it could be that antibody positive fisheries have never been exposed to the virus, but have received fish that have survived an infection at another site prior to movement and now no longer harbour the virus but retain immunity.

The route of introduction of the virus (or exposed fish) to a site is unclear in many cases, as in the case of antibody positive sites it is not known how long prior to testing that exposure may have occurred. Follow up sampling shows that fish remain antibody positive for at least one year after a clinical outbreak, however it is currently unknown whether these fish still carry the virus itself, or for how much longer this response may persist. Overall this study has provided valuable information on the distribution of KHV exposed fish in the UK, however laboratory work to investigate the risk posed by antibody positive fish to naïve stock would produce useful insight to the threat posed by the antibody positive sites identified in this study.

Many of the positive sites had received no consented movements of fish, however, although we have no firm evidence to support this; it is likely that some will have received un-consented movements. It is also possible that sites received fish without knowing it, possibly through hobbyists or anglers. Positive fisheries (both clinically and antibody positive) were more likely to hold both ornamental strains of carp and other ornamental species than sites testing negative. In many cases fishery owners stated that they did not know how these ornamental fish had been introduced to the site; it could be that hobbyists and anglers are largely responsible. In the majority of cases little is known about the origin of these ornamental species and it is possible that these fish have been reared in the UK and pose no greater threat than standard common carp.

Several suppliers of fish have been identified as supplying two or more positive fisheries and these sites require further testing. Of these sites, one was known to trade in vaccinated fish, and there were several clinical cases where this supplier of vaccinated fish had been the only known supplier to a case site. The potential for vaccinated fish to transmit the virus warrants further investigation, however the sites supplying these fish are responsible for many fish movements that occur each year, it is therefore likely that these sites will be associated with case sites by chance.

Although KHV exposed fish appear to be widespread throughout England and Wales, and prevalent within the fisheries sector, many sites remain free and our farming sector appears to be a relatively safe source of fish. Ornamental fish appear to play an important part in introducing the virus to the UK, however not all imported fish stocks have been exposed to the virus, and this route of introduction could be substantially reduced if importers are willing to test and quarantine stock, and deal with known safe suppliers. Education of hobbyists and anglers to the risks and consequences associated with introducing fish from the aquarium trade to fisheries is also critically important in order to prevent infected fish introducing the virus to our native stocks. Codes of good practice for both the farming and fisheries sectors are likely to be effective in reducing the spread of the disease if taken up by all or the majority of businesses within each sector. The incidence of the virus in our farms appears low and therefore the risk of introducing the virus to a fishery from a farm is considered low. Fishery to fishery movements appear to be higher risk as the status of many of our fisheries remains unknown and the amount of routine surveillance they receive is low in comparison to farms. In the cases of any movements of fish it is of great importance that the receiving site takes steps to ensure that the fish being moved have not been exposed to the virus and do originate from the source stated by the supplier. From our limited experience to date, it appears that clinical outbreaks rarely reoccur in the same sites in years following the initial outbreak. One possible explanation is that the virus does not easily persist after an outbreak, and it is therefore possible that imported fish are the source of most KHV outbreaks observed in the UK to date. The relative lack of persistence by the virus suggests that there is good potential to control the disease over time. This could be done through a combination of statutory controls, the use of codes of good practice, education and following a buyer beware policy.

Future Work

The current KHV distribution project has provided much of the data required by Defra in order to make informed decisions regarding the control of this virus. However, the study has been limited by the number of samples that it has been possible to collect from fisheries. This is due to the relatively short

sampling window, initial reluctance by the industry to participate in the study and the cold weather reducing sample sizes through low angler numbers and fish capture rate. As a result, it seems unlikely that the data obtained from the fisheries samples is sufficient to build a true picture of the regional status of fisheries with regard to KHV. In order to increase our knowledge in this area it is recommended that further monitoring of fisheries be conducted in 2008, predominantly focusing on high-risk fisheries and farms and those that have had contact with KHV infected sites. This will allow inference to be made as to the transmissibility and likelihood of expression of the virus in sites where known 'at risk' contacts have been made. Such work will also facilitate the identification of risk factors associated with KHV positive sites.

An increased understanding of the serological dynamics that occur in fish surviving KHV infections is another important area of research that is required in order to further validate the antibody testing being used, and to interpret the survey results correctly. Sera collected in the 2007 study from fisheries experiencing a KHV outbreak in 2006 showed an antibody positive response, however we do not know for how long this response may persist. Without such knowledge it is not possible to determine the maximum window within which the virus may have been introduced to a site, and as a result makes identifying possible source waters difficult. Additionally there is currently no robust information available to demonstrate the effect temperature/season has on the serological response present in fish or indeed how much time is required after an outbreak for a serological response to occur. This information is required in order to determine the test sensitivity at the time of sampling. To overcome these issues it is recommended that a small number of the 2006 outbreak sites be resampled in 2008 in order to determine if a response is still present. Additionally, it would be beneficial to obtain blood samples on a monthly basis for a year from a small number of sites in which cases occur in 2008 in order to determine when a response can first be observed and the effect temperature has on the diagnostic test sensitivity.

References

Denham, K. (2003). Koi herpesvirus in wild fish. *The Veterinary Record*, October 18th 2003, p507

Haenen, O., Way, K., Bergmann, S. and Ariel, E. (2004). The emergence of KHV and its significance to European aquaculture. *Bull. Eur.Ass.Fish Pathol.* 24(6) 293-307.

St-Hilaire, S., N. Beevers, K. Way, R.M. Le Deuff, P. Martin and C. Joiner, 2005. Reactivation of koi herpesvirus infections in common carp *Cyprinus carpio*. *Diseases of Aquatic Organisms*, 67: 15-23.

Way, K. (2004) Koi herpesvirus – A threat to wild carp. *Trout News* (Ellis & Glasscock eds.) CEFAS Lowestoft. 38: 32-34.

Way, K. and Dixon, P. (2007) Koi herpesvirus - An update. *Finfish News* (Ellis & Riches eds.) CEFAS Lowestoft, Issue No.3, 8-13.

Annex 1 – Letter sent to study sites requesting their participation in the KHV distribution study.

Dear

Research into the Distribution of Koi herpesvirus in England and Wales

I am writing to you to seek your co-operation in taking part in an important research project which we are undertaking to assess the possible distribution of the disease Koi herpesvirus in England and Wales.

As you may be aware, Koi herpesvirus (KHV) is a virus that can cause high rates of mortality in both coarse and ornamental carp (*Cyprinus carpio*). From the 6th April 2007 KHV disease became notifiable in the UK. This places a legal requirement on fish farmers, fishery owners and others with responsibility for the care of fish to inform Cefas if they suspect KHV in their fish stocks.

For many years there has been intensive trade in fish susceptible to KHV. As a result we do not know how widespread the virus is, and we therefore need to conduct research to identify what is possible in terms of control. In order to fulfil this research need, Cefas in collaboration with the University of Liverpool are conducting a research programme to increase our knowledge of the status of KHV in England and Wales. This work is being jointly funded by Defra and the UK's coarse and ornamental fish industry. The study aims to establish how common and widely distributed fish exposed to KHV are in England and Wales. Throughout the course of the study, fish from sites within different sectors of the coarse and ornamental industries as well as imported fish will be tested to see if they have been exposed to the virus.

Over the next few weeks we will be conducting a detailed field survey which will require the co-operation of fishery owners and managers. For this phase of the project we hope to take blood samples from fish from a representative range of fisheries throughout England and Wales. If you would be prepared to

participate in this research, a Cefas Fisheries Health Inspector will, by arrangement with you, visit the fishery and take a small blood sample at the waters edge from fish that anglers have caught. The fish will be unharmed and will then be returned to the angler's keep net or released.

Further details on KHV, the way we are conducting the research and what will happen to the results are included in the attached leaflet. Within the next few days, one of my colleagues will telephone you to discuss whether you would be prepared to participate in this project, answer any questions you might have and, if possible, arrange a site visit.

Your co-operation would be much appreciated and will provide important information which will help us manage and control this damaging disease.

Yours sincerely

Dr Nick Taylor
Project Leader

Annex 2 – KHV research fact sheet, sent to all sites contacted to take part in the KHV distribution study.

Research into the Distribution of Koi herpesvirus in England and Wales.

FACT SHEET

Koi herpesvirus (KHV) is a virus that can cause mortality in both coarse and ornamental carp (*Cyprinus carpio*). From the 6th April 2007 KHV disease became notifiable in the UK. This places a legal requirement on fish farmers, fishery owners and others with responsibility for the care of fish to inform Cefas if they suspect KHV in their fish stocks. For many years there has been intensive trade in fish susceptible to KHV. As a result we do not know how widespread the virus is, and we therefore need to conduct research to identify what is possible in terms of control. In order to fulfil this research need, Cefas in collaboration with the University of Liverpool are conducting a research programme to increase our knowledge of the status of KHV in England and Wales. This work is being jointly funded by Defra and the UK's coarse and ornamental fish industry and the Environment Agency. The study aims to establish how common and widely distributed fish exposed to KHV are in England and Wales. Throughout the course of the study, fish from sites within different sectors of the coarse and ornamental industries as well as imported fish will be tested to see if they have been exposed to the virus.

The following information is provided in response to frequently asked questions about the study, its findings and future controls that may be applied to sites with KHV.

Who are Cefas?

Cefas is the Centre for Environment, Fisheries and Aquaculture Science. We are an executive agency of Defra responsible for, among other things, the control of serious fish diseases in England and Wales.

What sort of test will the study use to screen fish for KHV?

Fish will be screened using a test that looks to see if a fish has produced antibodies in its blood to protect itself against infection by the virus. The test does not look for the virus itself.

Why is a test not being used that actually detects the virus?

Although there are test methods that can be used to detect the virus itself these have two problems: 1) fish must be killed for the sample to be taken, 2) fish that are carrying the virus at low level are difficult to detect using these methods.

If I give permission for samples to be taken do the fish used have to be killed? Not necessarily – in most cases as long as the fish are large enough (>15cm from tip of nose to base of tail) it should be possible to take the required amount of blood without killing the fish.

Do I have to allow my fish to be tested if asked?

We urge anyone approached to provide samples for KHV testing to take part in the project as without a thorough understanding of how widespread the virus may be, we cannot develop methods to control it. For us to tackle this disease effectively, it is important that we work together.

Does a positive result mean the fish are infected?

Not necessarily – as the test being used is not actually looking for the virus, it is possible that fish providing positive results are not infected.

Under what circumstances could fish give a positive test result but not have the virus?

- a) If the fish has been infected but managed to fight off and recover from the infection.
- b) If the fish has been vaccinated against the virus.

Can the test tell the difference between vaccinated fish and fish that have been exposed to the virus?

No – unfortunately by using the antibody test it is not possible to distinguish between vaccinated fish and those that are showing a response due to exposure to the virus.

Can fish become infected but not show an antibody response?

This is possible – as fish are cold blooded the effectiveness of their immune response is dependent on temperature. At low temperatures fish may be unable to produce a response if challenged to the virus and therefore could become infected but test negative using the antibody test. This is an area that has received little study, and further work is required to determine its potential significance.

Other than by the introduction of the virus, by what other routes could antibody positive fish get into my site?

If you have obtained vaccinated fish, they are likely to test antibody positive. In addition if you have received fish that have previously been infected but have since recovered from the disease, these are also likely to be antibody positive.

If a fish has been infected with KHV and recovered or has been vaccinated, how long will it show an antibody response for?

At present we do not know the maximum duration, but we have detected antibody several months after infection with KHV. Research is currently underway to provide an indication of how long after being vaccinated or recovering from infection fish can produce a detectable antibody response.

If my fish are tested as part of the study will I receive the results of the test?

Yes – when the samples have been processed we will notify the site providing the sample. However, as this is a research project, notification will not fall under the time limits normally applied by the Citizens' Charter when samples are taken for statutory purposes.

What are the current measures proposed for the control of KHV?

In summary, from the 6th April 2007 KHV disease became notifiable in the UK, meaning that Cefas must be informed if a site suspects clinical KHV disease. If the disease is confirmed a Designated Area Order (DAO) may be applied. Polymerase Chain Reaction PCR¹ is currently the definitive test for KHV and will be used to inform decisions regarding controls. The antibody test alone is not definitive and Cefas will not apply controls based on such a positive test result. The industry may however use this information for its internal codes of practice to inform trade and movement decisions.

If my fish test positive for KHV as part of this study, will statutory controls be placed on my site?

No – Cefas nor the Environment Agency will not apply restrictions to sites testing antibody positive for KHV as part of this study.

If I give permission for my site to be tested as part of this study will my site be identified in the dissemination of the results?

No – Results from all sites tested as part of the project will remain anonymous. At the end of the project when the results of the study are to be released the country will be divided into regions and only the proportion of sites testing positive within each region will be presented. We will ensure that each region contains sufficient sites that it will not be possible to identify which sites have been tested. No site names or locations will be presented.

If I get a negative result can I advertise my fish as KHV free?

No - although a negative test result is a good sign that your fish do not have or have not been exposed to KHV, the testing is indicative and for the reasons discussed in the above questions do not guarantee your fish are free from the virus.

¹ Polymerase chain reaction (PCR) is a technique which is used to amplify the number of copies of a specific region of DNA, in order to produce enough DNA to be adequately tested. This technique can be used to identify with a very high-probability, disease-causing viruses and bacteria.

Should the UK ban the import of fish from countries testing positive?

There are currently no specific EU import controls relating to KHV. This limits the ability of the UK Government to place restrictions on trade. Live fish imports do have to come from countries that are members of the OIE (The World Organisation for Animal Health) and that organisation now requires Member Countries to report and make public on its website outbreaks of KHV. . In addition, under EU rules it is a requirement that live fish are sourced from sites that have not suffered a clinical outbreak of disease within the last 6 months. That said, in many countries exporters themselves may not know the health status of the fish they are consigning in respect of KHV.

It is up to you to do what you can to establish whether or not the fish you are sourcing from imports are free of KHV. You may seek to source only from the most reputable exporters who may, for example, be prepared to provide assurances in commercial contracts that either the site or the batch of fish being sourced is free of KHV. In addition, or possibly as an alternative, you may decide to purchase fish from a UK based importer, wholesaler or dealer who as a policy of good bio-security practice requires fish sourced from abroad to undergo an acclimatisation process in isolated and controlled temperature condition to help identify latent KHV infection in the fish.

What other species of fish can carry KHV?

At present no other species of fish other than the coarse and ornamental varieties of carp – *Cyprinus carpio*, are known to carry the virus. However, to date relatively little research into this area has been conducted and there are currently several studies by different research institutions (including Cefas) investigating whether other species could harbour the virus.

When will further information from the study be released to the public?

Due to the complexities involved in interpreting the findings of this study no further results (other than the results of testing to sites providing samples) will be released until completion of the study in April 2008.

Who can I contact for further information about KHV?

Further information on KHV can be obtained through:

The Fish Health Inspectorate,
CEFAS Weymouth Laboratory,
Barrack Road,
Dorset, UK.
DT4 8UB

Tel: +44 (0) 1305 206600

Fax: +44 (0) 1305 206601

www.cefasc.co.uk

Alternatively updates and information will be posted on the eFishBusiness website:

www.efishbusiness.co.uk