

An assessment of the impact of importing carp (*Cyprinus carpio*) vaccinated against KHV on the site level prevalence of Koi Herpesvirus in England and Wales

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EXECUTIVE SUMMARY

A qualitative risk assessment was undertaken to investigate the likelihood that importing carp, vaccinated with an attenuated vaccine against koi herpesvirus (KHV), will increase the fishery and farm level prevalence of KHV infection in England and Wales at the current level of trade. Currently all imports of vaccinated carp into the England and Wales originate from Israel. The likelihood that the attenuated vaccine strain KV3 may revert to virulence was not considered in this analysis.

The guidelines for import risk analysis produced by the World Organisation for Animal Health (OIE) were followed. Scenario trees to illustrate the route of introduction and establishment for all three routes were developed. The steps necessary for the introduction and establishment of KHV via the importation of vaccinated carp were identified and individually assessed. Data on fish imports from Israel were analysed to assess current trade patterns. The large majority of imported carp are koi, destined for the ornamental trade. In comparison, significantly smaller numbers of common carp have been imported for release into fisheries. The relevant published data on KHV and in particular characteristics of the KHV vaccine were reviewed. It is known from experimental work and field observation that carp (*Cyprinus carpio*) may become sub-clinical carriers of KHV and, under certain conditions, the disease can recrudescence, resulting in re-excretion of the virus. Published data about the vaccine are sparse. However, the information available suggests that vaccination with attenuated KHV strain KV3 does not always prevent co-infection with wt-KHV.

It was concluded that the likelihood that an import of a single box of carp (based on average trade data for 2006, an average imported box contained 415 fish), would result in the introduction and establishment of wt-KHV was very low. A high level of uncertainty and natural variability was associated with a number of steps in the pathway, hence the final estimate also carries a high level of uncertainty. However, given the current volume of trade, it is likely that a number of outbreaks of KHV a year may be due to the importation of vaccinated carp (a quantitative analysis is required to provide a numerical estimate).

The importation and marketing of carp (common and koi) to the UK is complicated and crucial to the potential impact of vaccinated carp on KHV epidemiology in England and Wales. The large majority of carp are imported by ornamental wholesalers and destined

for garden ponds, but koi and ghost carp are also known to have been stocked into fisheries (in a recent survey it was found that 65% of fisheries had ghost or koi carp present). Common carp have been imported by carp farms. Imported fish are likely to pass through a farm to a fishery. Farms and cropping waters are potential distribution hubs, which may have an important role in the spread of KHV because imported vaccinated fish (infected with wt-KHV at time of importation) may introduce virus into the site, from where it could spread to other sites through the movement of live fish. The results of a recent survey, analysed by aquaculture business, showed that the proportion of KHV antibody positive sites is much lower among farms compared with fisheries¹. Therefore, importing potentially sub-clinically infected fish has greater potential to increase the farm level prevalence compared to the fishery level prevalence.

Since the risk assessment was completed, wt-KHV has been found in imported KV3 vaccinated koi (by PCR, confirmed by DNA amplicon sequencing) imported from Israel. It is highly likely that these carp were infected before arrival in the UK since two mortalities occurred in quarantine facilities. The outbreak supports the conclusion reached in the risk assessment that imported vaccinated carp may introduce wt-KHV. Well documented pathways (analysed in the risk assessment) exist which may result in exposure of carp in farms or fisheries to imported carp and, therefore, introduction of wt-KHV. The risk is exacerbated if vaccinated fish are marketed as, or perceived to be, 'safe' and therefore purchased by sites wishing to protect uninfected stocks.

¹ Farms breed carp to supply fisheries, dealers or retailers. Fisheries are managed stillwaters whose main source of income is the sale of fishing tickets; some fisheries may on occasion crop the water and sell surplus fish.

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1. INTRODUCTION

Koi herpesvirus (KHV) first emerged in Israel in 1998 (Gilad et al., 2002) and has subsequently spread to Europe, including the UK, and to North America and Asia (Hedrick et al., 2000; Haenen et al., 2004; Sano et al., 2004). Infection in common carp (*Cyprinus carpio*) may cause high levels of mortality when first introduced into a naïve population (Bretzinger et al., 1999; Hedrick et al., 2005).

An attenuated vaccine is currently available in Israel, where it is widely used in koi carp production, which are exported mainly to Europe and North America. Vaccine developments are also under way in several other countries (USA, Japan, Canada and Germany (Yasumoto et al., 2006; Fichtner et al., 2008)). We have no information whether fish destined for export from any of those countries are vaccinated prior to export. An immunisation procedure using exposure to wt-KHV and temperature manipulation is known to be practised in common carp produced for human consumption in Israel.

This paper assesses the likelihood that the importation from Israel of carp vaccinated against KHV results in a change in the prevalence of infection with wild type (wt) KHV in farms and fisheries in England and Wales, through the introduction, establishment and spread of wt-KHV virus. The importation of immunised common carp is also considered. The likelihood of reversion of the attenuated vaccine strain to high virulence is not considered. Risks of reversion to virulence depend primarily on the changes introduced into the virus genome at a molecular level (quality of mutation). Additionally, the likelihood that an attenuated virus may infect other fish species needs to be assessed. Other aspects of relevance are production standards during manufacturing (risk of contamination of the vaccine strain with wt- or other mutated virus strains; quality assurance of purity of vaccine strain). Currently, information available is insufficient to meaningfully undertake this assessment.

2. HAZARD AND RISK QUESTION

The pathogen hazard considered in this risk assessment is koi herpesvirus.

A qualitative risk assessment was undertaken to investigate the following question:

What is the likelihood that importing carp, either vaccinated with an attenuated vaccine, or immunised by exposure to wt-KHV followed by temperature manipulation, at the

current level of trade, will increase the fishery and farm level prevalence of infection with KHV in the England and Wales.

Currently, vaccinated fish are only imported from Israel.

3. ROUTES

The introduction of KHV vaccinated carp may increase the prevalence of KHV in England and Wales by three routes:

1. imported vaccinated koi (or common carp) are infected with wt-KHV when imported, and the virus is introduced to uninfected carp populations in England and Wales
2. imported vaccinated koi (or common carp) become infected with KHV (after introduction to England and Wales) and spread the wt virus to uninfected carp populations in England and Wales.
3. imported carp “immunised” using wt-KHV and exposed to temperature manipulation spread the wt virus to uninfected carp populations in England and Wales.

Route 1 was analysed further in the risk assessment. Route 2 does not result in the introduction of wt-KHV to the UK. Route 3 is potentially important but currently there is no evidence that carp ‘immunised’ by exposure to wt-KHV have been imported and is therefore not considered further.

4. RISK ASSESSMENT METHOD

The method for IRA recommended by the OIE was used (O.I.E., 2006). There are 4 stages to a complete import risk analysis (1) hazard identification, (2) risk assessment, (3) risk management and (4) risk communication. Risk assessment is further subdivided into: (1) release assessment (description of pathways necessary for introduction), (2) exposure assessment (description of pathways necessary for the exposure of aquatic species in the importing country to the introduced exotic pathogen), (3) consequence assessment (identification of the consequences of disease introduction and establishment) and (4) risk estimation (integration of the release, exposure and

consequence assessments) (O.I.E., 2003). Consequence assessment is not attempted in this risk assessment.

Qualitative risk assessment method

A scenario tree for route 1 was developed to identify the steps necessary for introduction (release) and establishment (exposure) of the virus for this route. Data requirements were identified (Appendix 2). Route 1 was investigated in depth through a qualitative risk assessment. Estimates of likelihood at each step were made (descriptions of the qualitative terms are given in Table 2) based on an importation of a single box of 415 juvenile carp. The method used in a recent EFSA report (Anon., 2007) was employed to combine qualitative estimates to produce an overall likelihood. Matrices are frequently used to combine qualitative estimates (see Vose (2000) and discussion for more detail). Using the matrix given in Table 2 the likelihoods of step 1 and step 2 were combined and this conditional likelihood was then combined with the likelihood of step 3, and so on. A high estimate followed by a high estimate gives a conditional moderate estimate of likelihood since the likely range of a high estimate will be between 0.7 and 1, with a median value of 0.8. Thus on average combining two high value results in a conditional estimate, which is moderate.

An estimate of the combined uncertainty (due to incomplete data) and variability (due to observed biological variation) associated with the risk estimate was also made (high, medium and low).

Table 1. Descriptions of combined uncertainty estimate

	Description
Low	Low level of observed biological variability OR A number of papers published in peer reviewed journals which provide consistent results
Medium	Moderate level of observed biological variability OR Limited data from only a few mainly non-peer reviewed sources or published data inconsistent
High	Very high level of observed biological variability OR No published data / only anecdotal reports.

Data on the current level of trade is used to estimate over a period of a year the likely change in site level prevalence due to the import of vaccinated carp.

Table 2. Description of terms used to describe the risk (Kahn et al., 1999)

Likelihood	Description
High (H)	expected to occur
Moderate (M)	occurrence less than 50% probability
Low (L)	unlikely to occur
Very low (VL)	rarely occur
Extremely low (EL)	very rarely occur
Negligible (N)	chance of occurrence so small it can be ignored

Table 3. Matrix used to produce the pathway conditional likelihood estimates

		Conditional likelihood to step 'n+1'					
		N	EL	VL	L	M	H
Likelihood at step 'n'	H	N	EL	VL	L	M	M
	M	N	EL	VL	VL	L	M
	L	N	EL	EL	VL	VL	L
	VL	N	N-EL ¹	EL	EL	VL	VL
	EL	N	N	N - EL ¹	EL	EL	EL
	N	N	N	N	N	N	N

H = high, M = moderate, L = low, VL = low, EL = extremely low, N = negligible

The shaded squares indicate the lowest possible likelihood (i.e. negligible)

¹N-EL combined with a likelihood equal or less than low reduces to negligible

5. BACKGROUND INFORMATION

Carp production in Israel

There are currently approximately 70 sites producing carp for food in Israel (Adrian Barnes, personal communication). It is estimated that five companies produce koi in

Israel, each of which have several sites. Business associations have changed over the last few years, with some sites going independent from the previous mother company.

KHV in Israel

The impact of KHV, following its first detection in 1998, on carp production in Israel was severe. Production fell steeply over a short period of time. Both carp produced for food and ornamental purposes were affected. The virus became widely distributed throughout the country. Exposure of juvenile stages to wt-KHV and water temperature manipulation were routinely used to avoid losses in larger fish.

An immunization procedure using exposure to wild type virus was authorized as an emergency measure in 2002. Subsequently, an attenuated vaccine has been produced (KV3 vaccine, Kovax). Currently there are two highly biosecure sites producing koi which claim to be KHV free and do not vaccinate. All other sites producing ornamental koi carp use the KV3 vaccine (Adrian Barnes, pers. communication). It is common practice to expose a small sample of vaccinated fish to wt-KHV to test vaccine efficacy. The evidence from Israel on vaccination of carp intended for food production is conflicting (see Appendix 1). It is most likely that KV3 is little used in this sector, due to its expense, and most farms use an immunisation procedure in juveniles based on challenge with wild type virus and water temperature manipulation (temporarily raised water temperature following exposure).

6. CARP IMPORTS FROM ISRAEL TO ENGLAND AND WALES

Data on carp imports from Israel to England and Wales are provided in Appendix 1. The data on fish imports to England and Wales from non-EU countries are limited. For this report import licences issued by the Fish Health Inspectorate (FHI, Cefas), and records held by the State Veterinary Service (SVS) of imports through the Border Inspection Post (BIP) at Heathrow airport were used.

Number of consignments imported

In 2006 a total of 238 consignments were imported from Israel through the BIP at Heathrow², that may have contained common carp or koi. Twenty-nine of these

² In recent years the majority of carp imports from Israel have been through Heathrow

consignments contained only *C. carpio*, all the remainder were mixed (e.g. with gold fish and tench). During 2006 the FHI inspectorate issued 8 single use licenses for the import of common carp for either aquaculture or restocking.

Numbers of fish imported

In 2006, the average number of fish per consignment was 15400 (minimum 31; maximum 111500, sd=17868). The average number of boxes per consignment was 42 (min 2, max 209, sd=35.9), and the calculated average number of fish per box was 415 (min 6, max 1394, sd=288). The total number of fish imported in consignments that were licensed for the import of common carp in 2006 was 100,686. It is not possible to estimate the number of koi carp imported but a total of 3.1 million fish were imported in consignments that could have contained koi.

Destination of imported fish

The majority of imports that may include carp (72%) went to ornamental wholesalers (Table 4)

Table 4. Imports of consignments from Israel into England and Wales in 2006 that may have included common carp or koi (BIP London Heathrow data).

Type of importer	Consignments	
	number	percentage
Carp farm	6	3
Combined ornamental wholesaler and carp farm	33	14
Mixed retailer	1	0
Ornamental wholesaler	171	72
Ornamental wholesaler and retailer	8	3
Ornamental retailer	14	6
Barramundi farm	3	1
Total	236	100

The vast majority of koi imported by ornamental wholesalers go into the ornamental trade (unpublished FHI data), however, a small amount of consignments also imported by ornamental wholesalers are delivered to fisheries or farm sites. The FHI has received anecdotal evidence of fisheries being supplied with ghost carp sourced directly from ornamental imports although this cannot be conclusively confirmed. Furthermore, some

aquatic retailers have separate businesses that sell some vaccinated carp through their ornamental outlet and some through their fishery business. Multi purpose sites (farm, fishery and ornamental units) have obtained vaccinated fish via ornamental wholesalers licences. The scale of this trade is generally considered to be small.

7. KHV IN ENGLAND AND WALES

A recent study (FC1180)³ estimated that the farm and fishery level prevalence of KHV (one or more seropositive fish) to be approximately 4% and 37%, respectively. High risk fisheries were sampled, hence the estimate may be higher than the national average. However, the sample size on many fisheries was low, which is likely to downwardly bias the fishery level prevalence estimate.

No KHV vaccine is currently licensed for use in the UK. KHV has been notifiable in the UK since 2007. Sites where clinical disease consistent with KHV and the virus is found are prevented from moving live fish, or stocking with susceptible species, for 12 months. Farms which rely on sales on live fish are likely to opt for de-stocking and disinfection in order that normal business can be resumed. KHV affected 23 fisheries in 2006, 10 in 2007 and 12 to date in 2008 (unpublished FHI data). The virus is also regularly detected in carp mortalities in garden ponds, retail outlets and importers' facilities.

8. BIOPHYSICAL CHARACTERISTICS OF KOI HERPESVIRUS

KHV titre declined in 3 days in environmental water or sediment from log 3.8 TCID/l to log 0.8 TCID/ml (Shimizu et al., 2006). A number of bacteria have been shown to have anti-KHV properties. The results suggest that KHV is rapidly inactivated and loses infectivity in environmental water (Shimizu et al., 2006).

It can be concluded that KHV does not accumulate in sediment or water and thus environmental reservoirs of infection are generally not important.

³ can be found at <http://www.efishbusiness.co.uk/news/080902a.pdf>

9. INFECTIOUS DOSE

Many authors have performed KHV infection trials by various routes including bath challenge, cohabitation, or injection in *Cyprinus carpio*. One of the lower challenge levels resulting in infection was 30 pfu KHV/ml water in a bath for 50 minutes (Pikarsky et al., 2004).

The minimum infectious dose can therefore be considered to be low, and therefore, low levels of virus excretion may lead to transmission..

10. VACCINE EFFICACY, IMMUNITY AND SUBCLINICAL INFECTIONS

The effect of water temperature

The koi herpesvirus disease is temperature dependent, occurring between 16–25°C (Hedrick et al., 2000). Under experimental conditions high mortality has been observed at 28°C (Gilad et al., 2004) but not at 29 or 30°C (Perelberg et al., 2005), nor at 13°C (Gilad et al., 2004). Viral DNA was detected in the fish by PCR at 13°C, and thus it is possible that infected fish surviving at low temperatures may be reservoirs of the virus (Gilad et al., 2004). It can be concluded that there is a permissive temperature range for disease expression between 14 and 28°C.

Age related immunity

Common carp and koi larvae (total length 7-9 mm) have been shown not to be susceptible to KHV (Ito et al., 2007). Furthermore, no evidence of KHV infection was found 21 days post challenge (all fish were negative by PCR). The same experimental conditions were used to challenge juveniles (total length 14-30 mm) with high resulting mortality (69% - 100%). None of the survivors was found to be KHV positive, all but one of the dead fish was KHV positive. This work suggests that larvae exposed to KHV do not become infected, do not develop immunity or die, but these same fish are susceptible as juveniles (and suffer high mortality). These results are reflected in the use of the KV3 vaccine in Israel, where experience has shown that vaccination before a critical age and size does not result in reliable immunisation.

Vaccine efficacy

The level of protection of carp against subsequent wt-KHV infection provided by vaccination with an attenuated vaccine strain depends on several factors. Very limited

information is currently available in the peer-reviewed literature. Perelberg et al. (2005) presented experimental data using the first and second generation live attenuated KHV vaccines, which provides the following information.

The development of a protective immune response in 10 g carp exposed to either a wt- or attenuated KHV strain appeared to depend on whether the virus propagated in exposed fish. Fish held at non-permissive water temperatures, either during exposure to an attenuated vaccine or immediately after, did not develop a protective immune response against wt-KHV associated disease. However, the permissive / non-permissive temperature range was not clearly defined. In the introduction, the authors mention that disease occurs at temperatures ranging from 18-28°C. Experimentally, 27°C was used as a temperature outside of the permissive temperature range. Furthermore, in 10g carp that were continuously kept at a permissive temperature during and after vaccination, protection provided by vaccination using attenuated vaccine against wt-KHV associated mortality was high, but not 100%. Experimental data showing the level of protection provided by vaccination in fish of different sizes is not provided.

Apart from the water temperature at which the bath vaccination was undertaken, the level of protection (measured in % survival of exposed fish) achieved by vaccination depended on the attenuated virus concentration in the bath (in a range of 0.1-100pfu/ml). Optimal protection against KHV associated mortalities was achieved at 10 and 100 pfu/ml vaccination dose.

The immersion time of naïve carp in a suspension of laboratory isolated wt-KHV (3 exposure times tested: 5, 15 and 40 minutes, at 200g fish / l) was positively correlated with the mortality rate. The authors concluded that immersion time is also positively correlated with prevalence of infection.

The experimental data presented by Perelberg et al. (2005) measured the level of protection achieved by attenuated vaccine as percentage survival of fish exposed to wt-KHV following vaccination. The authors did not investigate whether the survivors (that supposedly survived due to protection by the vaccine) became infected with wt-KHV.

More recently Perelberg et al (2008a) reported that approximately 90% of fish vaccinated survived challenge (sample sizes not given in the paper).

Currently no published peer reviewed information is available on

1. efficacy of vaccination in preventing co-infection with wt-KHV
2. duration of protection provided by vaccination with attenuated KHV
3. validation of experimental findings under farm or fishery conditions.

In the absence of peer-reviewed publications, the following assessments were made based on non-peer reviewed information and based on information available from related areas.

Does vaccination prevent co-infection with wt-KHV?

Ashoulin (2008) presented data on the experience of the Madan fish breeding centre in using an attenuated KHV-vaccine over the last 4 years: The relative percent survival (percent of vaccinated fish surviving a challenge with wt-KHV following vaccination) increased from 76%, in Koi, and 81%, in common carp, in 2004 to 92 and 91%, respectively, in 2007. These data very strongly suggest that not all carp mount a sufficient immune response to survive subsequent infection with wt-KHV.

Results presented by Siwicki et al (2008) reported similar findings. The lower the temperature at which the vaccination was undertaken (temperature range tested 16-22°C), the lower the protection achieved against subsequent challenge with wt-KHV (mortalities up to 80% in groups of carp vaccinated at 16°C following wt-KHV challenge).

It can, therefore, be concluded that not all carp vaccinated with an attenuated vaccine, can be considered fully protected against clinical wt-KHV infection. It is likely that in a vaccinated population, susceptible individuals exist. A proportion of these individuals will survive challenge with wt-KHV and of those a proportion will become sub-clinically and persistently infected with wt-KHV. Evidence for persistent sub-clinical infection is reviewed in the section below.

In further studies (Perelberg et al., 2008b), vaccinated carp were exposed to wt-KHV and then cohabitated with naïve carp. According to the data presented, the vaccinated carp did not transmit KHV to naïve fish. These results are in contrast with data provided by both Ashoulin (2008) and Siwicki (2008). The latter authors both reported mortalities due to wt-KHV challenge of previously vaccinated carp, whereas Perelberg

appears to not observe any KHV-related mortalities. The conflicting results may be due to different experimental conditions (data presented by Ashoulin (2008) and Siwicki et al. (2008) are from field observations, whereas data presented by Perelberg et al. (2008b) were obtained from experiments run under very controlled conditions). Details of the experimental conditions are difficult to extract from the information provided by Perelberg et al. (2008b)

In summary, it is likely that a proportion of fish from a batch of carp vaccinated with attenuated vaccine remains susceptible to wt-KHV infection. A batch of carp vaccinated with attenuated KHV vaccine should not be assumed to be free from wt-KHV infection. These conclusions are further supported by information provided by Mordi Haimi (MagNoy) during the 2008 KHV workshop in Israel (Haimi, personal communication). Mordi Haimi noted that co-infection of attenuated (vaccine) and wt-KHV had been detected and was not unusual. The same was reported recently by Sven Bergmann (national reference laboratory for fish diseases, Germany) at the meeting of the national reference laboratories, who had shown combined infection (wt and vaccine strain KHV) by PCR (Bergmann, personal communication).

Further evidence for the possibility of combined infections was presented by Meyer (2007). The author exposed carp to wt-KHV at permissive temperatures, then increased the water temperature up to non-permissive temperatures for 4 weeks and eventually brought water temperature back to the permissive temperature range (Meyer, 2007). Such fish developed disease when challenged orally with wt-KHV three months after returning to a permissive temperature (Meyer 2007), suggesting that the development of disease was associated with the second inoculation of KHV.

It can be concluded that co-infection with vaccine strain and wt-KHV in individual fish is possible. The likelihood of co-infection is likely to depend, *inter alia*, on the immune status of fish when exposed to wt-KHV.

Antibody response to vaccination and immunity

Perelberg et al (Perelberg et al., 2008a) found that approximately 90% of vaccinated fish survived challenge with wild type virus but the proportion of fish producing a measurable increase in antibodies was not provided. However, a marked increase in antibody (which developed 3-4 weeks post challenge) was shown to be associated with

protection against KHV disease when challenged shortly after vaccination, whereas fish with lower antibody levels remained susceptible (Perelberg et al., 2008a). Thus antibodies are likely to provide protection against wt-KHV infection, and it may be assumed that the antibody response in fish which did not survive challenge was very low or non-existent. If exposure to wt-KHV occurred shortly after vaccination, before a strong immune response was established, a higher likelihood of co-infection with wt-KHV is plausible. Similarly if exposure to wt-KHV occurred more than 12 months after vaccination, when immunity may have waned, persistent infection with wt-KHV at least in a proportion of animals may occur.

Conflicting information is available regarding co-infection of wt-KHV and attenuated virus. Both Perelberg (2008b) and Ashoulin (2008) postulate that vaccinated carp do not transmit either the vaccine virus strain or wt-KHV. In contrast, Mordi Haimi (see Appendix 5) based on field observations noted that co-infection of attenuated (vaccine) and wt-KHV had been detected and was not unusual.

In conclusion, persistent sub-clinical wt-KHV infection in vaccinated fish is plausible. The published evidence is not conclusive but the hypothesis is supported by field observations. No data exists on which to estimate the likelihood of persistent infection in vaccinated fish, but it is likely to depend, *inter alia*, on the immune status of the individual.

Duration of protection provided by vaccination with attenuated KHV strain against infection

The protection against wt-KHV infection in vaccinated carp is likely to rely on both humoral and cellular mechanisms. Immunoglobulin levels can be considered an indicator of protection and the available non-peer-reviewed data is reviewed in this section.

Steinitz et al. (2008) reported that antibody levels rise until 40 days post vaccination and then continuously fall to levels only slightly above those of naïve fish by 5 months after vaccination. Not all vaccinated fish developed a good antibody response. Information on temperature, fish size, or fish number in the experiments is not provided.

Cefas collected serum samples from carp in September 2007, originating from 3 fisheries holding carp that survived KHV outbreaks in June or July 2006 (unpublished

data). KHV antibodies were detectable in 88-93% of carp sampled (n=10, 25 and 30). Samples were collected in September, when the immune system is working well. Antibody levels were at levels that would be expected in recently infected fish.

Gangl (2008) found inconsistent antibody levels in koi that had recently survived an outbreak of KHV. The author took sequential samples from seven individual koi every 1-2 months over a period of 10 months. The fish were kept at water temperatures below the permissive temperature (8-18°C). In general OD values tended to be low and classification for KHV antibody levels in most fish was either “negative” or “questionable”. A great level of variation was found both between fish at a single sampling time point and within fish between sampling time points after exposure. A positive association between antibody levels and water temperature was observed. The fish were not stressed or challenged. Development of KHV-typical symptoms was not observed during the study period. It can be concluded that antibody levels vary greatly – with one determining factor being water temperature.

Another study suggests that antibody levels alone may not be a sufficient indicator of a good level of protection against clinical KHV. St.-Hilaire et al. (in press) measured antibody levels against KHV in a group of carp experimentally challenged with wt-KHV. Although medium to high levels of antibody against KHV were found in some fish, this did not appear to protect them against clinical disease. The fish had initially been exposed to wt-KHV at permissive temperatures, then cooled to below the permissive temperature range and eventually brought back into a permissive temperature range. Relatively high antibody levels were found during the low temperature phase. ELISA methods and interpretation of ELISA results may have varied between studies, and therefore the results may not be fully comparable.

Experience from wt-KHV outbreaks suggests that animals surviving a clinical outbreak of KHV are protected for a long time against developing clinical disease. This is likely to be based on a strong immune response (cellular and or humoral). However, whether a similar strength and duration of protection is induced following vaccination with attenuated vaccine is unclear.

Immunoglobulin levels found in carp that had survived an outbreak of KHV in the previous year (unpublished Cefas data mentioned above) were reasonably high,

suggesting that the immune system had been re-exposed to the antigen – possibly due to re-activation of a latent infection or virus circulating in the population. Therefore, a high level protection in survivors may be the result of continued exposure to the antigen.

Little is known about the “behaviour” of attenuated virus in the host. If the attenuated virus remains in a latent phase that is occasionally activated, a continuously high level of protection against clinical disease may be achieved. Data presented by Meyer (2007) suggest that a latent infection can be re-activated if the latent carrier carp was stressed.

In summary, no clear duration of protection against infection with wt-KHV or clinical disease due to vaccination can be provided. The level of protection is likely to vary with the temperature at which the vaccination was undertaken, age at vaccination, temperature at which animals were kept following vaccination and exposure to stress. Furthermore, absence of clinical disease is not a suitable indicator to conclude that an animal is not infected.

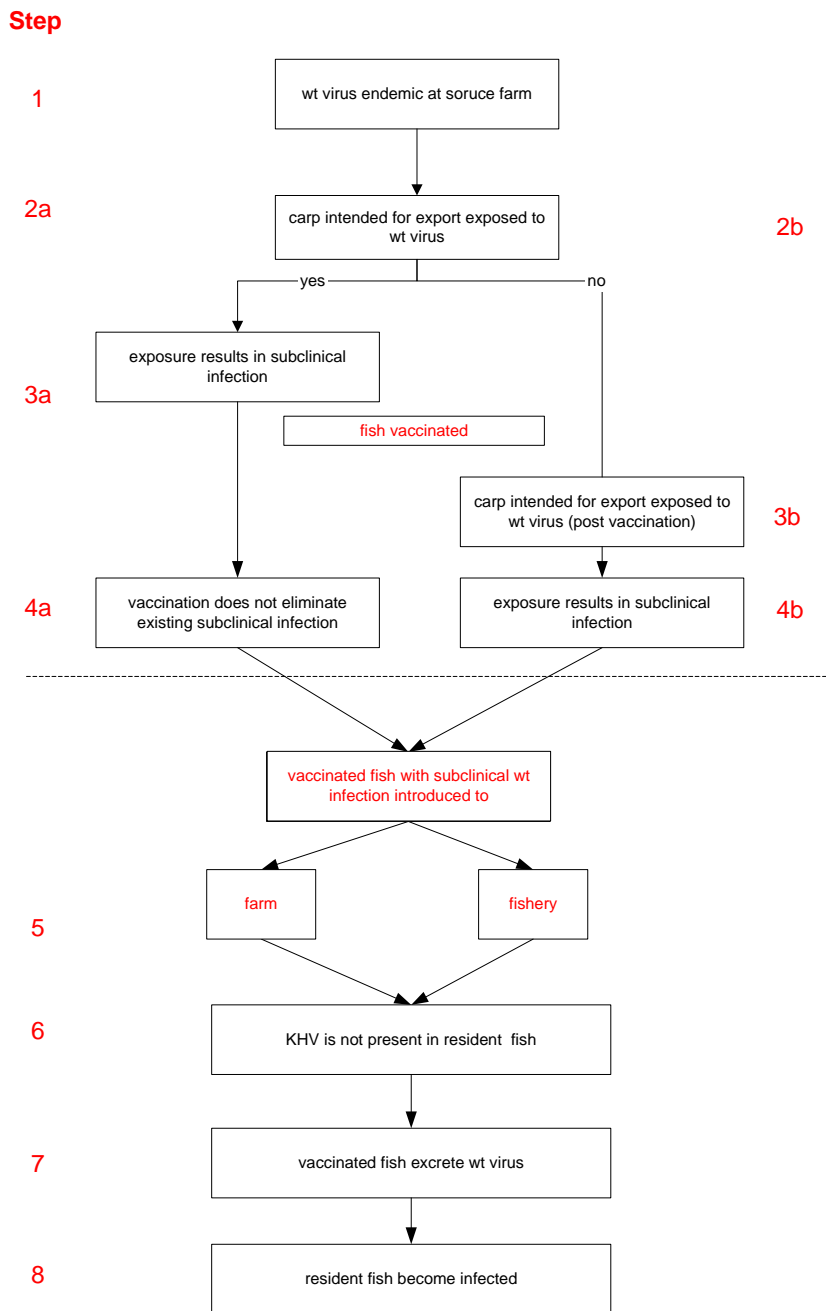
To what extent are experimental data transferable to the farm situation?

The studies discussed above (Perelberg et al., 2005; Perelberg et al., 2008b; Steinitz et al., 2008) used fish of specified size and defined temperatures. Vaccination was undertaken under controlled conditions and fish were isolated from wt-KHV infected animals for specified times. The conditions are, firstly, unlikely to be replicated on farms when vaccination is undertaken, and secondly, conditions will vary between farms and over time. The data presented by Ashoulin (2008), describing 4 years of experience with vaccination on Madan fish breeding center, demonstrated that a range of fish sizes were used and that the time period, for which fish are kept at a permissive temperature following exposure to the vaccine varied and could be shorter than suggested in experimental studies by Perelberg et al. (2005). Mortality rates presented by Ashoulin (2008) were significantly higher than suggested by work presented by others (Steinitz et al., 2008). Data from other farms are not available.

11. ROUTE 1 - VACCINATED CARP ARE INFECTED WITH WT-KHV WHEN IMPORTED, AND THE VIRUS IS INTRODUCED TO UNINFECTED CARP POPULATIONS IN ENGLAND AND WALES

The data requirements for this route are summarised in Appendix 2 and the scenario tree shown in Figure 1.

Figure 1. Scenario tree for Route 1



Step 1 wt-KHV endemic at source farm

Information

KHV became widely spread in carp production in Israel. All farms except 2 biosecure free sites vaccinate using KV3 or use natural challenge. Data presented by Haimi (see Appendix 5) suggest that farms are in close contact to each other and that transmission between sites is likely. It can be assumed that wt-KHV is present in all farms exporting koi to England and Wales, with the exception of the 2 sites known to be KHV free.

Likelihood estimate

High

Uncertainty estimate

Low

Step 2a Carp intended for export exposed to wt virus before vaccination

Information

Vaccination with KV3 takes place when the carp are 3-16g (at about 70 – 120 days of age; data: Madan fish breeding center (Ashoulin, 2008)). The likelihood of exposure to wt-KHV (and the level of exposure) will vary greatly between sites and depending on environmental conditions (e.g. water temperature). Some sites are developing biosecure wt-KHV virus free hatchery facilities where juveniles would be reared prior to vaccination (see appendix 1). One site (Ma'agan Michael) claims to have strict separation of naïve fish before vaccination and for a limited time after vaccination from wt-KHV infected fish. This site is otherwise not free from wt-KHV. Currently on most sites juveniles are not kept in biosecure facilities and therefore some level of exposure to wt-KHV prior to vaccination is possible, though high persistent challenge is likely to lead to high losses. No good evidence exists on which to estimate the likelihood.

Likelihood estimate

Moderate

Uncertainty estimate

High

Step 3a Exposure results in a persistent sub-clinical infection

Information

There is no good evidence on which to assess this step. In general the level of viral challenge is likely to be low. The minimum infectious dose for KHV has not been estimated but challenge with 30 pfu KHV/ml water in a bath for 50 minutes has been shown to be sufficient for transmission (Pikarsky et al., 2004). It has been demonstrated that infections with KHV can persist sub-clinically, and may recrudesce (St-Hilaire et al., 2005). It is estimated that in a consignment of 415 fish the probability of at least one fish being sub-clinically infected is medium.

Likelihood estimate

Medium

Uncertainty estimate

High

Step 4a Vaccination does not eliminate existing sub-clinical infection

Information

There is published evidence that co-infection of wt- and attenuated KHV can occur (Appendix 5). It has also been reported that carriers of wt-KHV are susceptible to re-infection if re-challenged with wt-KHV (Meyer, 2007). There is no available information from vaccine trials to indicate that vaccination eliminates a pre-existing wt infection.

Likelihood estimate

High

Uncertainty estimate

Medium

Step 5 Vaccinated fish introduced into a fishery or farm site

Transport is by air and it is therefore of short duration. It is assumed that there is no differential mortality rate between fish with and without sub-clinical wt-KHV infections.

The likelihoods are calculated for a consignment that is delivered directly (or indirectly via a wholesaler) to a fishery or farm, so this step has a probability of 100%. Data on patterns and volume of trade are used to assess the annual risk (see section on risk estimation).

Step 6 KHV not present at site of introduction

Information

KHV antibodies were found in 37% of sampled fisheries (FC1180 report). The study undertaken to investigate level of prevalence of previous exposure to KHV was biased towards fisheries with a high frequency of contacts. The overall prevalence is therefore likely to be lower than that suggested by the study results.

Likelihood estimate

Moderate - Fisheries; High – Farms.

Uncertainty estimate

Low

Step 7 Vaccinated fish excrete wt virus

Information

Studies on the potential release of wt-KHV from vaccinated fish that were exposed to wt-KHV before vaccination are currently not available. The likelihood of excretion of wt-KHV largely depends on whether these fish had been stressed and the level of stress (Meyer 2007). Given that the fish will have been transported to the site, a recent stressor would have been applied and excretion of wt-KHV would be likely.

Likelihood estimate

Moderate

Uncertainty estimate

High

Step 8 Resident fish become infected

The minimal infectious dose of KHV is very low. Stocking density in many fisheries is relatively high leading to a high contact rate. There is anecdotal information that introductions of single infected fish have led to an outbreak in a population of naïve carp.

Likelihood estimate

High

Uncertainty estimate

Moderate

Parallel branch for exposure post vaccination

Step 2b Carp intended for export NOT exposed to wt virus before vaccination

Information

See section above. It was estimated that there was a low likelihood that carp were exposed to wt virus prior to vaccination.

Likelihood estimate

High

Uncertainty estimate

Moderate

Step 3b Uninfected carp exposed to KHV post vaccination

Information

No good information on the likelihood of exposure of carp post vaccination is available. It is argued in step 2a that farms make efforts to keep carp unexposed to KHV for the

short period prior to vaccination. The perceived necessity for the same level of biosecurity post vaccination is less, since the fish have a degree of protection. Since KHV is present on the site it is assumed that there is a high likelihood of exposure post vaccination.

Likelihood estimate

High

Uncertainty estimate

High

Step 4b *Exposure results in sub-clinical infection*

Information

See section above (step 3a)

Likelihood estimate

Low

Uncertainty estimate

High

12. RESULTS

Table 5. Risk estimates¹ for route 1, exposure to wt-KHV prior to vaccination.

Step	Description	Risk estimate	Conditional risk estimate	Uncertainty estimate
	RELEASE			
1	wt-KHV at source farm	H	H	L
2a	Carp exposed to wt KHV	M	M	H
3a	Exposure results in sub-clinical infection	M	L	H
4a	Vaccination does not result in elimination of the wt infection	H	L	M
	EXPOSURE			
5	Vaccinated fish introduced into a fishery or farm	100%		
6	KHV not present at the site	H (farm) M(fishery)	L (farm) VL (fishery)	L
7	Vaccinated fish excrete wt virus	M	VL (farm) VL (fishery)	H
8	Resident fish become infected	H	VL (farm) VL (fishery)	M

¹Risk estimates based on one box of 415 fish

Table 6. Risk estimates¹ for route 1, exposure post vaccination, but prior to import.

Step	Description	Risk estimate	Conditional risk estimate	Uncertainty estimate
	<i>Pre-vaccination infection</i>			
	RELEASE			
1	wt-KHV at source farm	H	H	L
2b	Carp not exposed to wt-KHV prior to vaccination	H	M	M
3b	Carp exposed to wt-KHV post vaccination	H	M	H
4b	Exposure results in sub-clinical infection	L	VL	H
	EXPOSURE			
5	Vaccinated fish introduced into a fishery or farm	100%		
6	KHV not present at the site	H (farm) M (fishery)	VL (farm) VL (fishery)	M
7	Vaccinated fish excrete wt virus	M	VL (farm) VL (fishery)	H
8	Resident fish become infected	H	VL (farm) VL (fishery)	L

¹Risk estimates based on one box of 415 fish

Risk estimate

The overall likelihood of wt-KHV introduction is a function of the volume of trade and the risk per unit of trade. The overall likelihood that the importation of a single box of 415 carp from Israel to England and Wales resulted in the establishment of KHV in a carp fisheries and farms is very low (would only occur rarely). The likelihood of release is very low and the likelihood of exposure is moderate or low (for farm and fishery destinations respectively). However, given the current level of trade is high a number of KHV outbreaks may be due to the importation of vaccinated fish. The large bulk of the trade is koi carp imported by ornamental wholesalers (in 2006, 236 consignments – approximately 9900 boxes - may have contained koi carp, the number of imported koi carp cannot be estimated). It is likely that some of these koi enter fisheries via ornamental wholesalers. In addition a relatively small number of common carp are imported for release into fisheries (in 2006, 7 consignments – approximately 300 boxes - containing ca. 100,000 fish). The vaccine status of these fish is not known.

Given the current level and patterns of trade, import of vaccinated carp is likely to lead to a number of outbreaks of KHV each year in farms and fisheries in England and Wales, thus increasing the site level prevalence of infection. The KHV risk associated with purchasing vaccinated carp from Israel need to compared with purchasing carp from within the UK. Clinical KHV has never been detected in a population of farmed carp. Antibody positive carp were found in 4% of farmed carp populations (which may in part be attributed to the presence of vaccinated fish). In addition, KHV is notifiable and subject to a control programme (no live movements from sites where KHV disease has been detected). The KHV risks associated with purchasing carp from UK carp can be considered lower than purchasing imported vaccinated fish. A significantly higher level of antibody positive fish was found in fisheries, the sector where clinical disease is regularly found. Since persistent sub-clinical infections exist and clinical outbreaks may go unnoticed or unreported, it is not possible to conclude whether the purchase of carp from fisheries, compared with imported vaccinated fish, is a riskier option. It should be noted that fisheries may sell common but not koi carp.

Uncertainty

The steps in the pathway around which the highest level of uncertainty exists are the probability that, carp are exposed to KHV prior to vaccination, exposure results in sub-clinical infection, and, secondly, sub-clinically infected vaccinated fish excrete wt virus.

13. DISCUSSION

Key findings

The conclusion of the risk assessment was that the likelihood that a single box (415 fish) of vaccinated carp from Israel (route 1) results in introduction and establishment of KHV is very low. This is mainly influenced by the likelihood of release, which is very low. The likelihood of exposure was moderate or low depending on destination (farm or fishery, respectively); which indicates the probability of the hazard occurring if the consignment has infected fish. Given the volume of trade, a number of the outbreaks seen each year are likely to be due to the importation of vaccinated carp. The question arises whether replacing the current trade in imported vaccinated fish with unvaccinated fish decreases the likelihood that site level prevalence increases as a result of the importation. The import of unvaccinated koi or common carp from sources free of wt-KHV infection would not contribute to an increase of prevalence of KHV in cyprinid farms or fisheries. However, import of unvaccinated koi or common carp from endemically infected countries would also result in a number of KHV outbreaks and therefore an increase in the site level prevalence. No good evidence exists on which to judge the likelihood of persistent sub-clinical infection in vaccinated compared with unvaccinated koi, which have survived natural exposure to KHV. Therefore, it is not possible to estimate whether importing vaccinated or unvaccinated fish is a greater risk. It is concluded that purchase of carp from farms in the UK is less risky than purchasing imported vaccinated fish, but it is not possible to reasonably judge whether carp from UK fisheries present a higher or lower risk (see section above).

A danger exists that fisheries or farms, which have no history of KHV and consider themselves free of the virus, may introduce imported vaccinated fish because they are considered 'safe' (i.e. KHV negative), instead of buying from sources of known free status. The conclusion of this study is that it is not prudent, on the currently available evidence, to consider that vaccinated koi present no danger of spreading the virus. Marketing vaccinated carp as 'safe' (i.e. KHV negative) may lead to an increased site

level prevalence. By contrast, fisheries with a history of KHV disease or evidence of circulating virus (i.e. through sero-prevalence surveys) may consider vaccinated fish a safer purchase than naïve fish. However, the possibility exists for virus, introduced via vaccinated fish, to spread from an online fishery to downstream carp populations.

Importance of destination (farm, fishery or wholesaler)

The importation and marketing of carp (common and koi) from Israel to the UK is complicated. The majority of all carp from Israel are imported by ornamental wholesalers; nearly all of these will be koi. The large majority of koi are destined for the garden ponds, but koi or ghost carp are known to be stocked into some fisheries (and that ornamental wholesalers have supplied fisheries and farms). The scale of this trade is difficult to establish. In a study recently undertaken by Cefas (FC1180), it was found that 65% of fisheries had ghost or koi carp present (Taylor et al., 2008). Since the large majority of carp are imported through ornamental wholesalers, this is an important route even if the large majority of these fish are destined for ornamental use.

Common carp have been imported by carp farms. The business of farms is to sell live fish and, therefore, imported fish are likely to pass through a farm to a fishery. Many fisheries also crop and sell stock. In addition there are cropping waters, often associated with dealers, which are not fished. Farms and cropping waters are potential distribution hubs. Imported vaccinated fish may introduce virus into these sites, from where it may be spread to other sites through the movement of live fish. Data from a recent survey (project FC 1180) clearly indicates that farms have a considerably lower site level prevalence compared with fisheries. Therefore, importing potentially sub-clinically infected fish (route 1) has greater potential to increase the farm level prevalence compared to the fishery level prevalence.

Comparison of the routes of introduction

In route 2, the vaccinated koi become infected in England and Wales, which is more likely to occur in a fishery, compared with a farm. It is concluded therefore that route 1 presents a greater threat, compared to route 2, since this route is more likely to introduce virus into a farm, some of which have considerable potential to spread the virus through live fish movements. Carp immunised by exposure to wt-KHV (route 3) have probably a higher probability of persistent sub-clinical infection, compared with fish vaccinated with the attenuated strain, since exposure to the wt-KHV is certain. It is not known if

recrudescence of a sub-clinical infection is more likely in naturally vaccinated fish compared with a fish vaccinated with the attenuated strain.

Uncertainty and data availability

The report has highlighted important areas where our knowledge of KHV in vaccinated fish is deficient. Secondly, not all the published data are in agreement. The fundamental problem is a lack of published information about the vaccine, e.g. the duration of immunity and the protection against sub-clinical wt- infection. The risk of transmission of wt-KHV following introduction of vaccinated or immunised fish depends mainly on likelihood of recrudescence and excretion of the wt-virus. Little data exist on which to estimate these parameters. The other major area of uncertainty is the likelihood of exposure of carp to wt-KHV before or after vaccination. If the likelihood of exposure is extremely low (due to high levels of biosecurity), then the risk of introducing wt-KHV is similarly low.

The risk assessment method

The main advantages of applying a risk assessment approach is ensure that the necessary steps for the hazards to occur (in this case the introduction and establishment of KHV) are identified and logically ordered in a scenario tree. The necessary data to evaluate the hazard can then be identified and gaps in knowledge highlighted. There are clearly methodological limitations to the qualitative approach that has been adopted. Combining qualitative estimates is a problematic (Morris and Cogger, 2006) and the use of a risk matrix is not without drawbacks (see Cox (2008) for details). In this case it can be argued that the structure of the matrix underestimates the conditional likelihood because two steps with high likelihoods result in a medium likelihood, and two medium likelihoods results in an underestimation of the likelihood. However, conditional estimates of 'low' or 'very low' are propagated over a number of steps which results in an over-estimation of the likelihood. Despite its drawbacks, a qualitative risk assessment has provided a transparent basis for estimating the likelihood wt-KHV introduction and establishment. Critical steps necessary for the hazard to occur and paucity of information on which to estimate the likelihood have been identified.

A quantitative analysis is required to produce a numerical estimate of the likely increase in site level prevalence to KHV from continued importation of vaccinated carp from Israel. However, given the high level of uncertainty around many important parameters,

in particular data on the volume of imports, destination of imported carp and trade between fishery sites, any quantitative estimate would have wide confidence intervals. It is debatable whether a quantitative analysis would provide a significantly better basis for decision-making, compared with the qualitative result.

Other considerations

The existence of vaccinated carp in aquaculture production businesses complicates the interpretation of antibody surveys to establish prior exposure to the virus.

Conclusion

The current evidence suggests that fish vaccinated or immunised against KHV may be infected with wt-virus. It is known that recrudescence and excretion of the virus may take place. Insufficient data exists on which to estimate the probability that vaccinated / immunised fish may excrete wt-KHV. In comparing the relative risks of unvaccinated carp, immunised carp (by exposure to wt-KHV) and carp vaccinated with KV3, it is reasonable to assume that immunised fish are most likely to be sub-clinically infected. There is no evidence that common carp or koi imported to the UK have been immunised through deliberate exposure to wt-KHV. There are insufficient data to determine whether KV3 vaccinated fish or unvaccinated koi are more likely to be sub-clinically infected, having survived natural exposure. Pathways for the exposure of carp in fisheries and farms to vaccinated carp, and therefore wt-KHV, are well documented. The import of vaccinated fish is likely to be responsible for some of the KHV outbreaks observed in fisheries and result in an increase in site level KHV prevalence. The risk may be exacerbated if vaccinated fish are perceived to be, or marketed as, KHV free or 'safe', and thus may be introduced to fisheries or farms with no history of KHV that seek to protect this status by buying vaccinated fish.

14. POSTSCRIPT

Since the risk assessment was completed an outbreak of KHV has been investigated. Mortalities in garden ponds following the introduction of koi from one importer were noted. Koi at an importer's premises were visited (see Appendix 5 for details). Wild-type KHV was isolated from imported vaccinated koi, which had died in quarantine. These findings support the conclusion of the risk assessment that imported vaccinated carp introduce wt-KHV.

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16. APPENDIXES

Appendix 1 Carp imports from Israel to England and Wales

Limited records exist showing the number of fish imported into England and Wales from non-EU countries. The following two sources were available to analyse fish imports from Israel for this risk assessment: Firstly, electronic records held by the Fish Health Inspectorate at Cefas, on import licences issued and used. Secondly, paper records held by the State Veterinary Service (SVS) of cold and warm water species imported through the Border Inspection Post (BIP) at Heathrow airport. Imports on two types of licenses were analysed: **8A licences** allow the import of cold water ornamental species (koi and goldfish only) for a period of one year. Fish imported under this licence are destined to go into enclosed facilities. 8A licences are issued when the source of the fish is an approved SVC-free area. **8C licences** have a limited validity (2 months), are for single use only and for a specified purpose. Fish imported under an 8C licence can be any coldwater species, susceptible to SVC. An 8C licence is required, if

1. the fish are imported from a source that is declared free from SVC, and / or
2. the imported fish may be stocked into open waters.

Purpose categories for 8C licences are: aquaculture, ornamental, public display, restocking, research, or transshipping.

The paper records kept by the SVS sometimes specify the species imported, but more frequently summarise the content of a consignment as “coldwater” or “mixed” (coldwater and warm water fish mixed in the respective consignment).

Number of consignments imported

The paper records from Heathrow airport BIP and FHI data were analysed for 2006. A total of 271 consignments of fish provided by 9 different suppliers arrived in England and Wales from Israel in that year.

The Table below shows a summary of import data for 2006 of consignments imported through the BIP at Heathrow⁴, that may have contained common carp or koi. Further categories of fish imports that enter through the BIPs at Heathrow and Manchester airport, but are unlikely to have contained carp (and are for this reason not listed in the table below) are: Barramundi, goldfish, and “goldfish and 8C tench”.

Table A1.1. Number of consignments imported under an 8A, 8C or unclassified licence¹ from Israel into England and Wales in 2006 (BIP Heathrow airport data) that may have included carp or koi.

Category/ species	Licence type			Total
	8C	8A	8U ²	
8c fish	1			1
tench, koi, goldfish	1			1
Carp	2 (6) ³		2	4 (8) ³
Cw	26*	101		127
cw and 8c	11		1	12
Koi	6 (2) ³	19		25 (21) ³
koi and cw		1		1
koi and goldfish	1	4		5
Mixed	13	43		56
mixed and 8c	2 ⁴			2
Unknown	4			4
Total	67	168	3	238

¹Data are reported by categories as recorded in the London Heathrow BIP diary.

²8U= licence type not specified

³ 4 consignments officially recorded by the BIP to contain koi, may have indeed contained carp (8C license was issued for common carp).

⁴ one of these consignments was licensed for the import of common carp.

cw = coldwater

*koi, grass carp, tench or goldfish

The total number of consignments that may have contained carp is 238, of which 67 were imported under an 8C license and 168 under an 8A licence. The ornamental fish imports (imports under an 8A license) should theoretically not contain any common carp, but koi only. Imports under an 8C license authorise the import of fish for various purposes: aquaculture, ornamental, public display, restocking, research, or transshipping.

⁴ In recent years the majority of carp imports from Israel have been through Heathrow

In 2006, a total of 17 licences have been granted by the Fish Health Inspectorate for the import of common carp, of which only 7 appear to have been used.

In only 2 of those 7 used licences, did the paper records held at Heathrow airport match with the conditions of the licence. This explains the uncertainty about some of the data shown in the table below. Two further consignments, which according to Heathrow airport records contained carp, appear to have been imported without an 8C licence.

The above mentioned seven 8C licences specifically allowed the import of common carp for either aquaculture or restocking purposes. The remaining 60 8C licences were granted to import koi, goldfish, tench, grass carp for ornamental purposes, but not common carp. Since in many cases, the paper records from the border inspection post summarised the fish imports into categories, the exact number of koi imported cannot be established.

The reason for the requirement of an 8C licence is that either the respective fish species is also considered an aquaculture or fishery species, and /or, the import is to a site where the fish may be stocked into a farm or fishery.

In 2006, 8C licences for common carp imports were granted for one supplying site in Israel, which, according to official information provided to Cefas, vaccinated their common carp using attenuated vaccine. Koi imported under 8C or 8A licences have been provided by 7 different suppliers from an unknown number of sites. Two of the koi supplying sites now produce the fish under conditions where the stock is claimed to be free from wt-KHV (Hazardous Aquatics and Rift Valley). The exact date for the transition is not known to us. The remaining koi producing sites are assumed to vaccinate their koi using the attenuated KV3 vaccine.

The table below provides data for fish imports under 8C licences between 2000 and 2007. The data for the earlier years are likely to under-report the true number of imports under 8C licences.

Table A1.2. Fish imports under an 8C licence from Israel by year (FHI data)

YEAR	Number of consignments
2000	24
2001	18
2002	17
2003	14
2004	29
2005	53
2006	76
2007	62

Numbers of fish imported

In 2006, the average number of fish per consignment was 15400 (minimum 31; maximum 111500, sd 17868). The average number of boxes per consignment was 42 (min 2, max 209, sd=35.9), and the calculated average number of fish per box was 415 (min 6, max 1394, sd=288).

The total number of fish imported in consignments that were licensed for the import of common carp in 2006 was: 100,686. As mentioned above, the paper records at Heathrow airport were ambiguous for some of the consignments. The number of fish in consignments, which also according to Heathrow airport records clearly stated “carp” as the species imported would have been 50,110 imported under an 8C licence, plus 24,951, where the licence status was unclear.

Table A1.3. Number of fish imported under an 8A, 8C or unclassified licence from Israel into England and Wales in 2006¹ that included (or may have included) carp or koi.

Species	Licence type			Total
	8C	8A	8U	
8c fish	79,700			79,700
tench, koi, goldfish	4,175			4,175
Carp	50,110 (100,686) ²		(24,951)	75,061 (125,637)
Cw	664,698	1,071,101		1,735,799
cw and 8C	263,455		34,952	298,407
Koi	84,314	115,581		199,895
koi and cw		9,019		9,019
koi and goldfish	34,356	25,656		60,012
Mixed	193,331	513,607		706,938
mixed and 8C	58,595			58,595
Total	1,432,734	1,734,964	59,903	3,227,601

¹Data are reported by categories as recorded in the London Heathrow BIP diary. 8U= licence type not specified

² the number in brackets indicates the number of fish when summarising fish-numbers in all consignments licensed for the import of common carp under an 8C licence.

cw = coldwater

The number of koi imported – either under a 8C or 8A licence – is difficult to estimate, but could theoretically make up most of the fish imported under any of the “non carp” import categories (e.g. cold water or mixed); for fish imported under an 8C licence, this would be near 1.3 million fish and for fish imported under an 8A licence, up to 1.7 million fish (consignments not containing carp were not removed from Table 8).

Appendix 2. Data requirements for a risk assessment of Route 1.

Step	Description	Data requirements
	<i>Pre-vaccination infection</i>	
	RELEASE	
1a	wt-KHV at source farm	Farm level prevalence (wt KHV) in farms vaccinating fish before export
2a	Carp exposed to wt KHV	Management systems and biosecurity within farm. Age at vaccination.
3a	Exposure results in sub-clinical infection	Probability of outcome of exposure: no infection; infection resulting in death or recovery with or without sub-clinical infection (i.e. carrier). Outcome will depend on level of challenge, water temperature, immune status of the fish, age of the fish...
4a	Vaccination does not result in elimination of the wt infection	Experimental evidence from vaccine trials required.
	EXPOSURE	
5	Vaccinated fish are introduced into a fishery or farm, where KHV is not present on site	Destination of imported vaccinated koi. Fishery / farm level prevalence. Level of cross-protection between KHV strains.
6	Vaccinated fish excrete wt virus	Environmental conditions (temperature); immune status of the fish; level of stocking (leading to stress); nutrition. Experimental evidence from vaccine trials.
7	Resident fish become infected	Challenge level, minimum infectious dose, stocking density
	<i>POST-VACCINATION INFECTION</i>	
	RELEASE	
2b	Carp intended for export NOT exposed to wt virus	As for 2a.
3b	Carp exposed to virus after vaccination	Management of fish post-vaccination.
4b	Exposure results in sub-clinical infection in vaccinated fish	Experimental evidence from vaccination trials.
	EXPOSURE (as above)	

Appendix 3. Short report: Additional information from Israel to inform the risk assessment for koi or common carp vaccinated against KHV.

Keith Way

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1.1 Visit to Israel – Feb 16-19 2008

In 2007 the author of this report was invited by Moshe Kotler (Hebrew University-Hadassah Medical School) to a workshop on Cyprinid herpesvirus-3 (KHV) to be held in Caesarea in Israel in February 2008. The workshop mainly covered aspects of research on KHV and in particular research by Professor Kotler's students. This included persistence and expression of KHV in carp cells, potential latency in other cyprinid species and in immune carp and antibody response and resistance of immunized carp. Presentations by other Israeli fish biologists and vets included differential resistance of cross-bred carp to KHV, uncompromised biosecure production of koi and a clinician's perspective on 4 years of KV3 vaccination.

As well as being invited to present a keynote paper the workshop also included a very useful visit to the fish farm at the nearby Ma'agan Michael kibbutz. The Ma'agan site has now expanded to include the former Mayan Tsvi farm where the first outbreak of KHV disease occurred in Israel in 1998. In addition, I had also been invited to visit the indoor, 'biosecure', recirculation facility at Hazorea Aquatics before the workshop and to visit the Rift Valley Koi site in Galilee after the workshop.

2. INFORMATION TO ASSIST IN RESOLVING THE MAIN UNKNOWNNS.

2.1. The likelihood that farms exporting vaccinated carp to the UK has wild-type KHV (wt-KHV) endemic in the site (ie. farm level prevalence of wt-KHV).

I was informed by Yaniv Kotler (Kovax) that all sites producing ornamental carp (koi) use the KV3 vaccine. The prevalence of wt-KHV would be expected to be very low on these sites. He also stated that 80% of all Israeli carp production sites (ornamental & food carp) used the vaccine. The remaining 20% used no form of vaccination method.

Ofer Ashoulin (Vet at Ma'agan Michael fish farm) [Appendix abstract A1] stated that the efficacy of the immersion vaccine had improved over the last 3 years because improved culture systems have ensured that more carp are within the correct size range at the optimum age for immunisation. Relative percent survival (RPS) figures in 2007, when samples from different vaccinated batches of koi and common carp were challenged by co-habitation with carp infected with wt-KHV, averaged 92% for koi and 91% for common carp.

In contrast to the information from Yaniv Kotler, Avi Eldar (Vet at the Kimron Vet.Inst.) maintained that, because of the expense, only the ornamental carp sites used the KV3 vaccine. The food carp farms were still using exposure to wt-KHV to 'vaccinate' their carp. If this is correct then you would expect a higher prevalence of wt-KHV on these sites.

Furthermore, a presentation by Lior David (Faculty of Agricultural, Food & Environ. Quality Sciences, Rehovot) [Appendix abstract A2], on a genetic solution for KHV resistance in common carp, stated that producers and wholesalers in Israel use native and attenuated virus to immunize carp. Questions were also raised in this presentation about the long-term efficacy of the vaccine and the possibility that vaccinated carp could be carriers of wt-KHV.

Conclusion – It is very likely that a high proportion of farms exporting vaccinated carp to the UK have wild-type KHV (wt-KHV) endemic on their sites.

2.2 The likelihood that farms exporting vaccinated carp manage to keep their stock KHV free (assuming site is infected) before vaccination – this will depend on management and within site biosecurity.

At the Ma'agan Michael (MM) site carp are vaccinated by immersion at 4-10g and held for 4 days at optimal temperature for immunization. They are then held for a further 21 days in 'virus-free conditions' in water at sub-optimal temperature before being moved out to the ponds. The MM site now has a biosecure hatchery facility that has been in operation for 9-10 months. All juveniles are graded regularly in the hatchery. They have plans to install more tanks in the facility to enable them to vaccinate the juvenile koi within the facility before they are moved to the ponds. However, until vaccination is carried out within the facility, it is not clear if the farm can ensure that their juvenile

carp are virus-free before vaccination. Certainly the Israeli's maintain that there is a high level of 'herd immunity' and therefore the probability of juveniles being challenged with KHV is very low.

Conclusion - It is highly unlikely that farms exporting vaccinated carp manage to keep their stock KHV free before vaccination.

2.3 Does vaccination eliminate a pre-existing infection of KHV – is there any evidence from Israeli vaccination trials ?

In his presentation at the KHV workshop [Appendix abstract A1], Ofer Ashoulin reported that in numerous co-habitation tests with naïve fish, the vaccinated fish were found to be safe, whether or not they encountered wt-KHV prior to the test. However, these are not long-term safety trials and will not take into account carp that may have a latent KHV infection.

In the round-table discussion at the end of the workshop one of the questions raised was whether attenuated (vaccine) KHV and wt-KHV have been seen (detected) in the same fish. Mordi Haimi (MagNoy) confirmed that 'co-infection' had been detected and was not unusual.

Conclusion - vaccination does not ensure elimination of a pre-existing KHV infection.

2.4 Does exposure of vaccinated fish to KHV result in persistent infection (KHV carriers) ?

The short-term safety trials would indicate that vaccinated carp do not shed wt-KHV and transmit virus infection to naïve carp. However, this does not answer the question of virus persistence in these carp. It is unlikely that the Israeli's can answer this completely because they will not have situations where vaccinated carp are in close contact with naïve carp for extended periods.

At the workshop, Ayana Perelberg (Dor Aquaculture Research Station) presented studies on potential virus latency carried out with the Hebrew University [**Appendix abstract A3**]. This included a study to expose latency in immunized carp by thermal shock. They examined KHV-resistant carp previously exposed to attenuated KHV and survivors from a controlled infection with wt-KHV. After thermal shock wt-KHV did not re-emerge from either group of carp and could not be detected by PCR assay.

In trials carried out at Cefas, St-Hilaire et al. (2005) showed reactivation of KHV infections in common carp. However, groups of naïve fish cohabited with previously

infected fish did not always succumb to infection, and reactivation of virus did not occur, even following immunosuppression by cortisol injection. The authors considered that this was because none of the fish in those particular trial groups were persistently infected. These observations suggest that only a small percentage of carp become persistently infected following KHV infection and consequently the virus may only enter a latent state in a small percentage of carp.

Conclusion – If persistent infection can result from exposure of vaccinated fish to KHV is unclear. This may depend on the ability of KHV to enter latency.

3. OTHER INFORMATION

Danny Benjamin (Hazorea Aquatics) is avidly promoting biosecurity and in his presentation at the workshop [Appendix abstract A4] reminded the audience that vaccination can only be completely effective under biosecure conditions. At present there are only 2 sites in Israel that are producing koi in biosecure, KHVD-free conditions. Hazorea Aquatics, managed by Danny Benjamin, culture koi in an indoor ‘biosecure’ facility. Rift Valley Koi (RVK), co-owned by Adrian Barnes, produces koi on a relatively isolated, open site with a protected water source, a thermo-mineral water (28°C) spring. During my visit to Israel I visited both sites and was very impressed with the biosecurity measures they employ. Neither Hazorea or RVK use any form of vaccination against KHVD.

Andrzej ‘Kris’ Siwicki (IFI in Olsztyn, Poland) an immunologist, presented results of studies in Poland on vaccination of carp fingerlings with the KV3 vaccine [Appendix abstract A5]. He found that vaccine efficacy depended on route of administration of the vaccine and water temperature was very important. The highest mortality of 80% was observed in carp vaccinated at a temperature of 16°C and challenged with live virus at a temperature of 22°C. Whereas a mortality of only 20% was observed in carp vaccinated and later challenged at a temperature of 22°C. This work provides more evidence that successful vaccination against KHV can only be achieved in a controlled environment.

There was discussion outside the workshop on the role of fish-eating birds in the spread of KHVD. The Ma’agan Michael farm in particular, because of its situation on the coast, has persistent problems of predation by cormorant and gulls. Israel is also on a

pelican migration route from Eastern Asia to Africa and huge flocks of these birds fly through the country in the late spring and summer. A flock of pelicans that are able to alight on a pond can eat all of the fish in the pond in a very short time. The flock then flies on and many of the birds that have overeaten will regurgitate fish that fall into nearby ponds on that site or on neighbouring sites. On the Ma'agan Michael site many of the smaller ponds are netted and a gas cannon is used to scare birds from the larger ponds. However, the site is limited in its use of bird deterrents because it lies within a national park where the birds are protected. If KHV is prevalent on farms rearing carp for food, because they are not using the KV3 vaccine, then this represents a source of wt-KHV that could easily be spread by birds. Farms rearing food carp are commonly found in close proximity to sites that also culture koi carp.

Appendix 4. Selected abstracts of presentations at the KHV workshop (Israel, February 2008)

A1 - KV3, FOUR YEARS OF VACCINATION – A CLINICIAN’S PERSPECTIVE

Ofer Ashoulin (DVM) , Madan, Maagan Michael Kibbutz, Israel

Since the devastating outbreak of Koi Herpesvirus disease in 1998, there has been dramatic progress in our ability to control the disease.

Vaccination of koi and common carp with KV-3, an attenuated live CyHV-3, has become the standard tool in the battle against KHV at the Maagan Michael fish farm. During the years 2004 – 2007, KoVax and Maagan Michael have collaborated in vaccinating millions of fish in different protocols, until finally a safe product was achieved, which consistently results in RPS ≥ 85 and RPS ≥ 95 in immersion versus injection, respectively.

In our search for the procedure that will best fit the conditions of the farm, we had to take into consideration the adverse effects of excessive handling of koi. Due to the high prevalence of Aeromonas-related lesions following booster immersions 3 weeks apart, we had to provide a one step solution, which we finally did. In numerous cohabitation tests with naïve fish, the vaccinated fish were found to be safe, whether or not they encountered the wild type virus prior to the test. The presentation will focus on the conditions found to be critical for successful vaccination.

A2 - TOWARDS A GENETIC SOLUTION FOR CyHV-3 (KHV) RESISTANCE IN THE COMMON CARP

Gideon Hulata^a, Lior David^b & Yaniv Palti^c

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The magnitude of damage caused to the carp industry by the CyHV-3 virus, requires a multi-dimensional approach to achieve a sustainable solution. The current state allows producers and wholesalers to immunize fish using native or attenuated virus in order to minimize animal loss. Although effective, immunization and vaccination are not free of difficulties and concerns. More for edible carp and less for koi, the cost of the vaccine and vaccination process is not negligible.

Moreover, the longer-term efficacy of the vaccine is yet to be assessed. In addition, as of now, the infection cycle of the virus is not fully understood and thus fish, including other cyprinids, once exposed to the virus, might be hosts for future outbreaks. Here we propose to embark on a longer-term process to produce genetically resistant carp. We will begin with estimating the genetic variability of KHV resistance by testing all possible crosses between three strains of carp, namely the susceptible Dor-70 and Našice, and the wild, resistant Sassan.

By comparing survival rates and serum antibody titers between progeny groups, we will infer the type and magnitude of genetic variability as well as the preferable type of breeding program that might increase carp resistance. From susceptible and resistant F₁ progeny, we will produce genetically polymorphic F₂ and backcross progeny groups for molecular diagnosis. In parallel, we will develop several hundred DNA markers, mostly microsatellites, for the carp. The genotypic data will be analyzed for genetic linkage between markers to construct a second-generation genetic map. Individuals from the polymorphic progeny groups will be phenotyped for their resistance and genotyped for the alleles of each DNA marker. Similarly, genetic linkage between phenotype and DNA markers will be analyzed to identify QTL that control viral resistance in carp. In addition, several genes known to play a role in the immune system of fish will be cloned and examined for their involvement in viral resistance of carp. Either DNA markers or specific genes that are associated with resistance will in the future be used for marker assisted breeding of carp resistant to CyHV-3.

We maintain that genetically resistant fish will be a much more cost-effective, environmentally safe and sustainable solution to this and possibly other viral infections of both edible and ornamental carps.

A3 - POTENTIAL LATENCY OF CyHV-3 IN THREE FISH SPECIES OF CYPRINIDAE AND IN IMMUNE KOI CARP.

Avana Perelberg¹, Moshe Kotler², Arnon Dishon², Itzhak Bejerano¹

¹ Dor Aquaculture Research Station, Fisheries Department of the Ministry of Agriculture, Israel; ² Department of Pathology, The Hebrew University–Hadassah Medical School, Jerusalem, Israel.

Since its first outbreak in 1998 in Israeli carp farms, CyHV-3 disease has been characterized by seasonal reappearance, independent of attempts for controlling it. Spontaneous outbreaks occurred in autumn and spring, with no evidence during winter and summer. The question "where is the viral agent preserved between seasons" is still actual. One of the possible explanations is latency in the carriers' tissues. This possibility could be extremely significant to

the koi carp industry worldwide. During 2006, we carried out a preliminary trial series in order to reveal latency potential in fish. The first experiment was aimed to reveal potential latency in 3 fish species, all belonging to the Cyprinidae family, and common to Israeli aquaculture: *Carrasius auratus*, *Hypophthalmichthys molitrix* and *Myelopharingodon piceus*.

The sensitivity of these species to CyHV-3 and their possible role as vectors were assessed by a series of controlled infections and exposures *in vivo*:

1. Direct infection by injecting w.t CyHV-3 i.p., and by cohabitation with infected koi carp (*Cyprinus carpio*) to assess sensitivity of the 3 cyprinid species to the disease.
2. Incubating injected fish of the 3 cyprinid species with naïve koi carp in permissive conditions, to assess transmission of the virus by cohabitation.
3. Injecting naïve koi carp with tissue filtrate prepared from any of the fish species infected before.
4. Incubating kidney filtrate prepared from any of the fish species in CCB tissue culture.

Fish tissues were sampled for PCR assay in each stage of the experiment. The results show no evidence to latency of CyHV-3 in fish of the 3 species examined.

The second experiment was aimed to disclose latency in immunized resistant koi carp, by exposing them to thermal shock. We then asked two questions:

1. Will thermal shock following incubation in the permissive condition cause a change in the immunological status of immunized resistant koi, and leave them susceptible to a challenged infection.
2. Will thermal shock lead to a relapse of the virus in immunized and resistant fish, permitting spontaneous infection and morbidity or immunization among naïve fish in the same water system.

Two types of resistant fish were examined: fish that were vaccinated by attenuated CyHV-3 virus, and fish that survived a controlled infection with wild type CyHV-3. Thermal shock induced mortality due to parasite infestation in fish that were exposed to it, but in accordance with negative PCR results, there was no evidence for virus relapse and no effect on their sensitivity to the viral disease. The results also show that attenuated CyHV-3 is not spontaneously transmitted from vaccinated to naïve fish.

A4 - THE CASE FOR UNCOMPROMISED BIOSECURE PRODUCTION OF KOI FOR THE ORNAMENTAL FISH INDUSTRY

Daniel Benjamin² & Adrian Barnes¹

¹ Rift Valley Koi, Kibbutz Mevo Hama, Israel; ² Hazorea Aquatics, Kibbutz Hazorea, Israel.

Koi Herpesvirus (KHV) under suitable environmental conditions results in heavy mortalities of populations of Koi and carp (*Cyprinus Carpio*). koi are traded on a continuous basis in the worldwide ornamental fish market and the common carp is an important protein source in many countries. During the last decade KHV has spread rapidly throughout the world and has resulted in severe economic losses to Carp and Ornamental fish industries in many countries.

In Israel, KHV has been documented since 1998 and three "solutions" to the problem have since been offered by Israeli companies, all claiming to help in the containment and eradication of KHV.

- Initially, many farms in Israel performed natural immunisation (NIS) of the carp or koi they bred and sold.
- Recently a vaccine has been developed and is used in several koi and carp producing farms in Israel.
- Certain farms in Israel have destroyed all infected or compromised stock and continue production in indoor or outdoor virus free, Biosecure facilities.

The paper will present the case for KHV free production of non-compromised Koi on bio-secure farms and facilities in the context of a strategy of worldwide eradication and containment of KHV. The basic principles of biosecure fish production will be discussed and the implications of the different methods of production practised in Israel to the International trade of koi and carp will be examined and compared.

A5 - EFFECTIVENESS OF KOVAX (ISRAEL) VACCINE AGAINST CyHV-3 IN CARP FINGERLINGS (*CYPRINUS CARPIO*) – EXPERIMENTAL STUDY IN POLAND

Andrzej K. Siwicki¹, Yaniv Kotler², Barbara Kazuń¹, Edward Głąbski¹, Krzysztof Kazuń¹, Elżbieta Terech-Majewska³.

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The protection of carp against CyHV-3 by immunization has been an important concept for a number of years. One of the most common uncertainties regarding the use of vaccines is how long the protection will last. In our study we concentrated on improving the vaccine's potency and efficacy, and optimally activating cell-mediated immunity and specific immune response. In the first part of the study KoVax vaccine (1ml of vaccine diluted in 10 L of water) + 1 kg carp fingerlings weighing 10 g each was used by immersion. The immersion time was 40 minutes at a temperature of 22°C. After 21 days 100 fish were experimentally infected by injection with CyHV isolated from Polish carp; 100 fish were experimentally infected by injection with CyHV-3 isolated in Israel; 100 fish were experimentally infected by immersion with CyHV-3 isolated from Polish carp and 100 fish were experimentally infected by immersion with CyHV-3 isolated in Israel. The first control group consisted of fish not vaccinated and infected with either Polish or Israel virus, and second control group consisted of fish merely vaccinated. The fish were maintained in 7 tanks of 500 L (100 fish per tank) and were fed daily with commercial pellets. Mortalities were tabulated and controlled, and the presence of pathogen was confirmed by isolation from the gills and pronephros. In the second part of this study KoVax vaccine (1ml of vaccine diluted in 10 L of water) + 1 kg carp fingerlings weighing 10 g each was used by immersion. The immersion time was 40 minutes at temperatures of 16°C, 18°C, 20°C and 22°C. After 21 days 50 fish from each temperature were experimentally infected by injection with CyHV-3 isolated from Polish carp at a similar temperature, and 50 fish from each group at 22°C. The control group was fish which had only been vaccinated. The fish were maintained in tanks of 500 L (100 fish per tank) and were fed daily with commercial pellets. Mortalities were tabulated and controlled, and the presence of pathogen was confirmed by isolation from the gills and pronephros.

The results of the first part of the study showed that the percentage of mortality after a challenge test by injection was higher, compared with challenge tests by immersion and was

similar for Polish and Israeli viruses. The highest mortality was by injection: 25% with the Israeli virus and 22% with the Polish virus, compared to the first control group where mortality was 98% with Polish virus and 95% with Israeli virus. The mortality after challenge by immersion was 15% with the Polish virus and 10% with the Israeli virus.

The results of the second part of my lecture show that the temperature during vaccination is very important for inducing high protection against CyHV-3. The highest mortality was observed in fish vaccinated at a temperature of 16°C and infected by live virus at a temperature of 22°C (80%), compared to the group of fish vaccinated at a temperature of 18°C (68%) and 40% in fish vaccinated at a temperature of 20°C. Fish vaccinated and infected at similar temperatures were protected against CyHV-3 differently. At temperatures of 22°C mortality was lowest (20%), compared to temperatures of 16°C (32%), 18°C (40%), 20°C (38%).

Appendix 5. Koi herpesvirus outbreak in imported vaccinated koi (September 2008)

In September 2008 the owner of an import/retail centre specialising in the sale of aquatic animals contacted the Fish Health inspectorate after a number of his customers reported mortality in their carp. The mortalities were seen in the resident koi after introduction of koi carp from the retail centre. Clinical signs were indicative of KHVD. A fish health inspector visited the main site receiving imported fish. Koi in all systems were generally healthy but two dead koi (KV3 vaccinated and imported from Israel) were found and sampled. Both koi were from quarantine systems, one from an indoor and the other from an outdoor system. Tests on both fish produced strong positive signals from single-round KHV PCR assays. This strength of positive signal is expected in samples from carp with clinical KHV disease. Further PCR assays and sequencing confirmed the DNA amplicon as wild type KHV (wt-KHV). More recently, virus isolation has been confirmed from tissue homogenates inoculated onto common carp brain (CCB) cells.

Further samples were taken from healthy ghost koi purchased directly from a UK producer (2 pools, 5 fish per pool) and held in an indoor system. Samples were also taken from imported vaccinated koi (from the same overseas source) in an indoor system (2x 6 fish pools) and an outdoor system (2x 5 fish pools). In a nested KHV PCR assay, the ghost koi tested negative for KHV and the two groups of imported carp both tested positive. However, because of the low levels of KHV DNA in these samples, no discrimination of vaccine and wt-KHV was possible from further tests.

Further testing was undertaken at second site, owned by the same business. Samples were taken from imported vaccinated koi showing possible KHV disease signs (2 groups, 4 fish per group). Gill tissue extracts from both groups tested KHV positive in a nested PCR assay. Further PCR assays and sequencing confirmed the DNA amplicon as wild type KHV (wt-KHV). There was some evidence that more than one strain of wt-KHV may have been present.

More recently (2 September), during a visit to the second site to supervise clearance and disinfection, further samples were taken from diseased vaccinated koi from a recent shipment. On this occasion, tissue extracts tested KHV positive in a single-round PCR

assay and sequencing again confirmed the DNA amplicon as wt-KHV. The virus is also growing in CCB cell cultures inoculated with tissue homogenates.

It is likely that the mortalities reported by customers of this retailer were due to KHV. High levels of wt-KHV DNA were found in two dead carp at the main site and much lower levels in healthy koi from the same batch. High levels of wt-KHV DNA were also found in koi showing disease signs at a second site. No vaccine strain KHV has been detected in any of the samples. Samples from both sites have also been found positive in virus isolation tests. The evidence indicates that vaccinated koi were infected with KHV. Since the fish were held in quarantine ponds, it is most probable that they became infected before export to the UK. It can be concluded that the infection recrudesced, resulting in disease and virus excretion. The outbreak demonstrates that the vaccine does not provide 100% protection.