

# Transmission of Koi Herpesvirus via angling equipment

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## Introduction

Koi herpesvirus (KHV) is a fatal disease of carp (*Cyprinus carpio*) which has spread rapidly between recreational fisheries in the UK. Movement of infected carp presents a major risk of spreading KHV disease, but it is possible transfer may also occur via contaminated angling equipment such as nets (Figure 1). With little direct proof of transmission via this route, a series of simple studies were designed to investigate its potential.



Figure 1. carp being held in a keepnet (top) and gill necrosis typical of KHV disease (bottom).

## Experiment 1: Transmission of KHV via anglers' nets

- KHV diseased carp (Figure 1) were held in two fine mesh keep nets overnight at 23°C.
- Infected carp were then removed and naïve carp introduced either immediately or after the net had been stored for 24 hours in a sealed plastic bag.
- Both groups of naïve carp displayed clinical signs on KHVD within 14 days of transfer to contaminated netting.
- The study demonstrated that under ideal conditions KHV could be transmitted via anglers' nets.

### Risk assessment

- Although KHV has the potential to be transmitted in this way, a risk assessment is required to understand the level of risk.
- Our study aimed to work towards defining and quantifying the risk pathway associated with transmitting KHV on anglers' nets (Figure. 2).
- A review of popular angling literature revealed that 87.5 % of UK fisheries stock carp, and of these 52.4 % allow use of keepnets and all allow landing nets.
- These data suggest a high proportion of carp fisheries are potentially at risk of transmission via this route.
- The following tank and laboratory studies were conducted with the aim of quantifying the probability associated with important steps in the risk pathway.

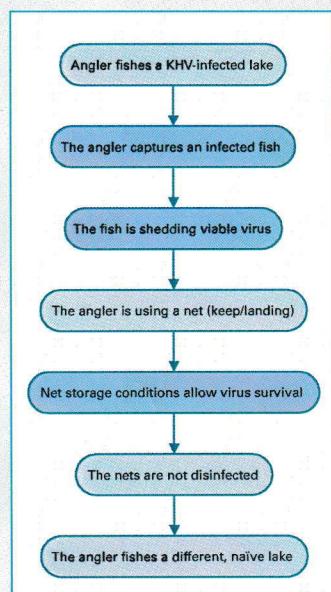


Figure 2. Risk pathway for transmission of KHV on angling equipment. Steps highlighted in darker blue were the focus of tank and lab trials.

## Experiment 2: Feeding behaviour vs. virus shedding

- To investigate the likelihood of an angler catching a fish shedding KHV, the relationship between feeding and virus shedding was examined.
- Twenty common carp were bath challenged with KHV and five controls mock challenged with culture medium. These were separated into individual static tanks at 23°C and fed 40 pellets of food each day. Feeding rate was monitored daily by removing and counting any uneaten pellets. Carp were netted from their tank each day and replaced. Mucus transferred to the nets was then tested using Taqman real time PCR to quantify levels of virus shed. Any survivors were tested for anti-KHV antibody using ELISA.

### Results

- All of the carp exposed to KHV became infected and 75 % developed clinical disease.
- The remaining 25 % mounted a successful antibody response and survived the infection.
- Virus shedding began on the first day post-exposure and continued throughout the trial.
- Though fish challenged with KHV fed at a significantly lower rate to controls, they still ate over 40 % of food presented to them, whilst shedding virus at levels of  $1 \times 10^8$  copies ml<sup>-1</sup> mucus.
- The results show that fish shedding virus are still likely to be caught and therefore have the potential to contaminate netting.

Table 1. Number and proportion of flasks of CCB cells positive for KHV-induced CPE.

Conditions	Dark and Damp	Dark and Dry	Light and Damp	Light and Dry
KHV positive	15	3	0	0
KHV negative	0	12	15	15
Cytotoxic	0	0	0	0
Proportion positive for KHV	100 %	20 %	0 %	0 %

## Experiment 3: The influence of net storage conditions on virus survival

- Experiments 1 and 2 established that KHV infected carp continue feeding whilst shedding virus and that, under ideal conditions, nets contaminated by such fish could transfer virus to naïve fish.
- This study aimed to assess how different net storage conditions that may be encountered between fishing trips, alter the likelihood of transmission.
- Carp mucus spiked with KHV was used to coat strips of net which were placed in clear plastic zip-lock bags and kept under one of four conditions: 1) dark and damp, 2) dark and dry, 3) light and damp and 4) light and dry. Dark samples were kept in an opaque box, light samples were exposed to sunlight, damp nets were sealed in their plastic bag, whilst bags for the dry samples were split open. The nets were incubated for 18 hours before mucus was removed (and if required, rehydrated with water), centrifuged and filtered (0.45μm) prior to inoculating common carp brain (CCB) cells. The cells were then monitored for development of KHV-specific cytopathic effect (CPE).

### Results

- KHV survived best in dark/damp conditions and less well in dark/dry conditions (Table 1).
- KHV did not survive exposure to sunlight.
- Drying reduces KHV infectivity but is only completely destroyed by exposure to sunlight.
- Anglers that dry their nets (and other equipment) and expose them to several hours of sunlight prior to using them, minimise the risk of transferring KHV by this route.

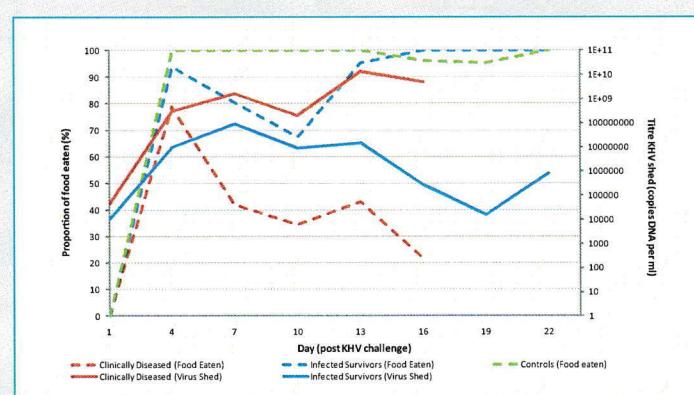


Figure 3. Relationship between percentage food eaten by carp and copies virus DNA shed per ml mucus for 22 days post KHV-challenge.

## Summary

- KHV has spread rapidly through recreational carp fisheries in the UK.
- This study has demonstrated that anglers' nets may have played a role in this rapid spread.
- Carp infected with, and shedding KHV continue feeding and therefore have the potential to be caught by anglers.
- A high proportion of UK carp fisheries allow fish to be held in nets.
- If fish held are infected they are capable of contaminating nets to a level that allows transmission of KHV to naïve fish.
- Anglers drying and exposing their nets to sunlight can substantially reduce the risk of transferring the virus to other waters.
- Compared to live fish movements the risk transmitting KHV on anglers' nets is low, however the high frequency at which such contacts occur provide the potential for nets to have a substantial influence on the spread of the pathogen.