Koi herpesvirus (KHV) causes a severe disease and mass mortalities in populations of common carp (Cyprinus carpio carpio) and koi carp (Cyprinus carpio koi). The disease has spread rapidly around the world and devastating losses have occurred in intensive and extensive carp culture facilities in Europe, Asia and North America. The disease is now notifiable to the World Organisation for Animal Health (Office International des Epizooties, OIE).

The behaviour of the virus in the host makes it very difficult to confirm the presence/absence of the virus in apparently healthy fish with a latent herpesvirus infection. Studies in Israel and the USA have identified serum antibodies to KHV in carp that have survived disease outbreaks (1, 2). The antibody response is consistently present in survivors and it persists for a long period of time, it may be possible to use KHV-specific antibodies to identify fish populations that have been exposed to the virus. However, antibody assays must be validated and the prevalence and duration of antibodies in fish should be determined.

This study evaluated an ELISA, developed in our laboratory, for detection of serum anti-KHV antibodies in populations of experimentally exposed common carp.

Materials and Methods

The fish used in this study were part of another study to evaluate KHV persistence in common carp (3). The same tank identification system used in the published study is maintained here for continuity.

Fish and tanks - Common carp were obtained from a farm in England with no previous history of KHV. All fish tanks were on a flow-through system with 2 L min⁻¹ of de-chlorinated bore-hole water. Fish were fed a maintenance diet of 1% bodyweight d⁻¹ and monitored 2 times d⁻¹.

Viruses - KHV (isolate UK D-132 and Cyprinid Herpesvirus-1 (CyHV-1) isolate F-265). Both viruses were grown in no.1 fin (KFR-1) cells as described previously (4). For the ELISA, viruses were purified on sucrose density gradients (approx. 10⁴ pfu ml⁻¹ of virus for 2 h in a static bath at a temperature of -9°C).

Fish were transferred to a new tank (2A) and the temperature in this tank was maintained at 18°C for 2 d and then subsequently the temperature was lowered to 11°C to ensure some fish survived the viral outbreak. Surviving fish in tanks 2A and 3B were used to monitor the antibody response, and both tanks were subject to the temperature profile shown in Figure 2.

2nd KHV exposure trial - 30 common carp (25 to 30 g) were injected IP with approx. 10 pfu KHV in 0.2 ml of FBBA and returned to a tank (3B) with 500 common carp to serve as the source of virus. 100 carp were maintained in a separate control tank (3C) and used to monitor the antibody response as the course of the study (2nd KHV exposure) and all fish were negative for antibodies.

Figure 2: Cumulative mortality rate and seroprevalence of fish is tanks 2A and 3B exposed to KHV and maintained at an elevated temperature of 18°C for three weeks. The temperature profile is included on the secondary Y axis. Seventy seven control Fish in tank 2A were tested for KHV antibodies over the course of the study (2nd KHV exposure) and all fish were negative for antibodies.

Serum collection and handling - Blood was drawn from the caudal vein of carp, after they had received a lethal dose of anaesthetic, 2ml non-irradiated Vacutainer® (BD Biosciences). Blood was allowed to clot overnight at 4°C and serum harvested and stored at -20°C until tested for KHV-specific antibodies.

Antibodies to KHV were not detected in all exposed fish sampled in our study suggesting that this test is more useful in identifying the exposure status of the population rather than individual fish. Negative results when fish are sampled at only one point in time may be inconclusive. To avoid this, screening programs can be progressive. The assays can be used on a large scale to provide evidence of the KHV exposure history. Also, if the fish are large enough (i.e. >25 g) the sample could be collected non-lethally.

The time required for an antibody response to be detectable in the carp, post-exposure to the virus, was longer in this study (between 10 and 19 weeks) than the 3 to 6 weeks reported by other workers. This was almost certainly influenced by the temperature manipulations and further studies at Cefas have shown detectably higher levels of antibodies will be present in carp at 3 weeks post-exposure when held at a constant 22°C.

There appears to be little cross-reaction with CyHV-1 antibodies at high serum dilutions but only ten fish were investigated in this study. Carp with antibodies to KHV were not detected in the koi used for cross-reactivity studies with the homologous virus, CyHV-1 instead of KHV.

The results of this study indicate that carp populations with persistently infected fish (virus carrier fish) have a high seroconversion rate and seropositive prevalence of KHV detectable antibodies. Our results support those from previous studies (12) demonstrating that the ELISA provides a useful non-lethal tool for identifying fish populations that may have been persistently exposed to the virus and are not showing clinical signs of disease.

References

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Development of a serological test for koi herpesvirus surveillance

Results

Anti-KHV antibodies were detected in all populations of virus exposed fish regardless of whether the population experienced high initial mortalities or not (Figures 2 and 3). There was no evidence of anti-KHV antibodies in fish from populations of the control groups not exposed to the virus. In the experimental groups, antibodies to KHV were first noted at 10 (tank 2C) and 19 weeks (tank 3B) post-exposure and antibodies were still detectable at high titres (≥1:1600) in 25% of the fish after 25 weeks (25). All fish considered seropositive in this study had titres at a serum dilution of 1:1600 or greater. Elevated mortality associated with reactivation of KHV occurred in tanks 2C and 2D (at 20 weeks post-exposure) and in 3B (at 25 weeks post-exposure) during a high proportion of antibody positive fish in the populations (Figures 1 and 2).

Six of the 10 koi exposed to CyHV-1 had detectable antibodies for anti-CyHV-1 antibodies had no detectable anti-KHV antibodies by ELISA. Four fish with antibody titre to CyHV-1 of 1:1600 or greater (carp #1, 4, 6, and 7) had cross-reactions with KHV at lower serum dilutions (1:20 and 1:400).

Discussion

Following exposures to KHV, a high proportion of fish in the two populations produced detectable antibodies to KHV. There was a reduction in the number of fish with detectable antibodies over time but after 25 weeks 25% of the carp in tank 2D were seropositive (titre 1:1600) (Figure 1). Also, regardless of whether fish were maintained at a high or low temperature or experienced high virus-associated mortality, a large proportion of the fish seroconverted (Figures 1 and 2).

Antibodies to KHV were not detected in all exposed fish sampled in our study suggesting that this test is more useful in identifying the exposure status of the population rather than individual fish. Negative results when fish are sampled at only one point in time may be inconclusive. To avoid this, screening programs can be progressive. The assays can be used on a large scale to provide evidence of the KHV exposure history. Also, if the fish are large enough (i.e. >25 g) the sample could be collected non-lethally.

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There appears to be little cross-reaction with CyHV-1 antibodies at high serum dilutions but only ten fish were investigated in this study. Carp with antibodies to KHV were not detected in the cross-reaction for CyHV-1 and this needs to be investigated further. To validate this assay, naturally infected populations need to be evaluated. Results of tests on populations of common carp and koi carp that have survived KHV disease outbreaks are summarised in Table 1.

Table 1: Detection of antibody to KHV by ELISA in populations of koi and common carp that have survived natural KHV infections and in carp vaccinated against KHV disease

<table>
<thead>
<tr>
<th>Site</th>
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<td>Vacinated</td>
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</tr>
<tr>
<td>B</td>
<td>Farm</td>
<td>March 2006</td>
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<td>C</td>
<td>Koi dealer</td>
<td>June 2007</td>
<td>Survivors of KHV</td>
<td>9 of 42</td>
</tr>
</tbody>
</table>

The results of this study indicate that carp populations with persistently infected fish (virus carrier fish) have a high seroconversion rate and seropositive prevalence of KHV detectable antibodies. Our results support those from previous studies demonstrating that the ELISA provides a useful non-lethal tool for identifying fish populations that may have been persistently exposed to the virus and are not showing clinical signs of disease.