Infection with koi herpesvirus-Disease Card

by

Mark Crane, Motohiko Sano and Cedric Komar

Pathogen Information

1. Causative Agent
   1.1. Pathogen Type
       DNA herpes-like virus
   1.2. Disease Name and Synonyms
       Infection with koi herpesvirus
       Carp nephritis and gill necrosis
       Koi mass mortality
   1.3. Pathogen Common Name and Synonyms
       Koi herpesvirus (KHV)
       Carp nephritis and gill necrosis virus (CNGV)
   1.4. Taxonomic Affiliation
       Unclassified
   1.5. Description of the Pathogen
       The aetiologic agent was first isolated by Hedrick et al. (2000) and, based on ultrastructure, was named koi herpesvirus. Subsequently, following further disease outbreaks in Israel, it has been suggested that the virus should be named carp nephritis and gill necrosis virus (CNGV) (Ronen et al., 2003). At the International Workshop on koi Herpesvirus, London, February 2004 (Anon., 2004a), it was agreed that these names refer to the same agent as well as the causative agent of the mass mortality of koi and carp reported in the Asian region (Anon., 2004; c.f. Way et al., 2004).
   1.6. Authority
       In 1997 and 1998, mass mortalities, associated with the presence of a herpes-like virus, occurred in koi carp from several countries including, Germany, UK, the Netherlands, Israel and USA (Bretzinger et al., 1999). The virus was first isolated in cultures of KF-1 cell line (Hedrick et al., 2000). Samples were taken...
from adult koi (*Cyprinus carpio*) that were maintained in ponds or tanks in the USA and Israel and were suffering mass mortalities.

1.7. *Pathogen Environment*

   Freshwater

2. Modes of Transmission

2.1. *Routes of Transmission*

   Horizontal

2.2. *Life Cycle*

   The origin of the virus is not fully understood. However, it is proven that the infection is transmitted horizontally from other infected fish, such as introduced carriers of infection (Bercovier et al., 2004).

2.3. *Associated Factors*

   The disease is highly contagious and cumulative mortality can approach 100%. Temperature is an important factor. Moving infected fish from 13°C to 23°C results in rapid onset of mortality (Gilad et al., 2003). In addition, secondary gill infections (for example, *Flavobacterium columnare* and *Aeromonas* spp.) are often associated with KHV infection.

2.4. *Additional Comments*

   Table 1 summarises the sensitivity of the virus to various physico-chemical conditions. Further research is required in this area.

**Table 1. Summary of viral sensitivity to physico-chemical conditions**

<table>
<thead>
<tr>
<th>RESISTANCE TO PHYSICAL AND CHEMICAL ACTION</th>
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<tbody>
<tr>
<td>Temperature</td>
<td>Infectivity destroyed after 2 days at 35°C (Neukirch, 2003). As a guideline, until further research is undertaken, 60°C for 30 minutes for virus inactivation.</td>
</tr>
<tr>
<td>pH</td>
<td>Infectivity destroyed at pH &lt;3 or &gt;11 (Neukirch, 2003)</td>
</tr>
<tr>
<td>Chemicals</td>
<td>Sensitive to chloroform (and presumably to other lipid solvents and oxidising agents – research required for confirmation)</td>
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<tr>
<td>Disinfection</td>
<td>As a guideline, until further research is undertaken, disinfection should be carried out as recommended by the OIE (2003).</td>
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<tr>
<td>Survival</td>
<td>Virus can survive for at least 20 hours in water and probably longer in mud ponds – further research required.</td>
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3. Host Range

3.1. *Host Type*

   Common and koi carp

3.2. *Host Scientific Names*
Cyprinus carpio

3.3. Other Known or Suspected Hosts

None

3.4. Affected Life Stage

All age classes; the disease has occurred in fingerling, juvenile and adult common and koi carp.

3.5. Additional Comments

Japanese researchers, during an outbreak in the spring of 2004, recorded that the outbreak occurred in wild carp populations at water temperatures >15-16°C, and most of the dead fish were adult. In the field, it appears that adult carp are more susceptible than juveniles.

Permissive temperature range: up to 28°C. While the virus may survive at low temperatures (5°C), the temperature range for disease outbreaks appears to be 17-28°C.

Mortality due to infection of other freshwater fish species, such as goldfish (Carassius auratus), grass carp (Ctenopharyngodon idellus), silver carp (Hypophthalmichthys molitrix), silver perch (Bidyanus bidyanus) and tilapia (Oreochromis niloticus), by this virus has not been observed naturally or by experimental infection (Perelberg et al., 2003). To date, the disease is species specific.

4. Geographic Distribution

4.1. Region

The disease and agent have been reported from North America, Asia, Europe, Africa and the Middle East.

4.2. Country

In USA, the virus was first isolated in 1997/8 (Hedrick et al., 2000). Outbreaks of disease with signs similar to infection with KHV were first reported from UK in 1998 with virus isolated in 2000. In Israel, KHV was first diagnosed in 1998 and reported in 1999 (Ariav et al., 1999). The current known geographical range of the disease is shown in table 2.

Table 2. List of countries and dates of first reports of KHV

<table>
<thead>
<tr>
<th>Europe</th>
<th>America</th>
<th>Asia</th>
<th>Africa</th>
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<tbody>
<tr>
<td>Switzerland</td>
<td>USA (2003)</td>
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<td>Luxemburg</td>
<td>USA (2003)</td>
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<td>Italy</td>
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<td>Austria</td>
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<tr>
<td>France</td>
<td>USA (2003)</td>
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</table>
4.3. Additional Comments
It is suspected that, due to the worldwide volume of the ornamental fish trade, the actual geographical range is wider than the known geographical range. The distribution is not, yet, worldwide but covers a wide range of continents.

Disease Information
1. Clinical Signs and Case Description
1.1. Host Tissues and Infected Organs
Lesions are observed in, and virus can be isolated from, gill tissue, fresh kidney, liver and spleen indicating that the infection is systemic.

1.2. Gross Observations and Macroscopic Lesions
- Pale/irregular coloration of the gills and skin
- Severe gill necrosis
- Superficial branchial and skin haemorrhages
- Occasionally sunken eyes and congestion of fins are observed
Examples of signs of disease are shown in figures 1 and 2.

1.3. Microscopic Lesions and Tissue Abnormality
- By light microscopy, hyperplasia and fusion of secondary gill lamellae are observed (figure 3). Intranuclear inclusions in the branchial epithelium may be observed. Necrosis of liver, spleen and kidney parenchymal cells can be observed.
- By electron microscopy, intranuclear inclusions containing herpes-like viral particles are observed. (N.B. In the cases in Taiwan (Tu et al., 2004) and Japan, the intranuclear inclusions were rarely found).

1.4. OIE Status
- Infection with KHV is not listed by the OIE

2. Social and Economic Significance
Cyprinus carpio is raised as a foodfish (common carp) in many countries and ornamental varieties (koi) are popular as pets with hobbyists and as exhibition fish. The value of individual ornamental koi can be as high as US$several thousand. Production of carp aquaculture worldwide is valued at US$several billion per annum.

3. Zoonotic Importance
To date, no zoonotic reports exist.

4. Diagnostic Methods
Different diagnostic procedures exist but, to date, an agreed standard diagnostic procedure has not been developed. Currently, several methods are in the process of being ring-tested among laboratories specialised in KHV diagnosis. Following this evaluation, it is anticipated that a recommended standard diagnostic procedure will be established.
4.1. Screening Methods

Detection of carriers is a major issue. For quarantine, participants at the International koi Herpesvirus Workshop, London, 12-13 Feb. 2004 (Anon., 2004a) recommended that rearing fish at 23-28°C for a minimum of 2 weeks (3-4 weeks would be better) followed by PCR check for KHV can be useful for detection of carrier fish. The procedure should be performed at the time of both exporting and importing.

This principle is used for the testing of koi carp before exhibitions: Due to the highly contagious nature of the disease co-habitation can be used to determine whether very expensive koi carp are infected. Since koi carp are very expensive lethal sampling is not appropriate. Thus prior to importation of koi carp the koi are placed in quarantine tanks together with (cheaper) common carp that were demonstrated free of KHV. After 2 weeks at 23-28°C, the common carp are sacrificed and tested for KHV. If these fish are free from the disease, then the more expensive koi are considered free as well.

4.1.1. Level I

There are no diagnostic signs exhibited by sub-clinical carriers.

4.1.2. Level II

Histopathological lesions in sub-clinical carriers are not detectable.

4.1.3. Level III

Virus isolation in cell culture does not appear to be reliable. Detection of carriers by PCR may be more reliable than virus isolation but is subject to well-established caveats.

4.2. Presumptive Methods

4.2.1. Level I

Disorientation, erratic swimming behaviour, mass mortality, discoloration and severe necrosis of the gills are indicative of infection with KHV.

4.2.2. Level II

By light microscopy, hyperplasia and fusion of secondary gill lamellae are observed. Intranuclear inclusions in the branchial epithelium may be observed. Necrosis of liver, spleen and kidney parenchymal cells can be observed.

4.2.3. Level III

By electron microscopy, intranuclear inclusions containing herpes-like viral particles are observed. (N.B. In the cases from Taiwan (Tu et al., 2004) and Japan, intranuclear inclusions were rarely found).

4.3. Confirmatory Methods

4.3.1. Level I

There are no pathognomonic signs of infection with KHV.

4.3.2. Level II

Differentiation from Herpesvirus cyprini (Carp herpesvirus 1 (CHV)) and other gill diseases responsible for gill necrosis such as Flavobacterium columnare infection is required.
4.3.3. Level III

4.3.3.1. Virus Isolation

For virus isolation, gill tissue, fresh kidney and spleen from acutely infected or moribund animals should be sampled. However, it should be noted that once gill tissue has been infected by KHV it becomes necrotic and very often develops secondary bacterial infections (for example, *Flavobacterium columnare* or *Aeromonas* spp.). Therefore it is more difficult to isolate virus from necrotic gill tissue and the cell culture medium becomes contaminated by bacteria even with the use of antibiotics. Therefore, it may be preferable to isolate this virus from internal organs and especially kidney. Virus isolation, using fresh tissues from moribund fish, can be achieved using KF-1 (or common carp brain (CCB)) cell cultures (Hedrick et al. 2000; Neukirch & Kunz, 2001). Virus isolation from frozen tissues can be unreliable. This method is not suitable for detection of carriers. Even using moribund or freshly dead fish, virus isolation can sometimes fail (because of low susceptibility of the cells).

4.3.3.2. Nucleic Acid Assay

Currently, any one of several available polymerase chain reaction (PCR) assays can be used (Gilad et al., 2002; Gray et al., 2002; Bercovier et al., 2004). For PCR, fresh or frozen gill tissue, kidney and spleen from moribund or freshly dead fish. In addition, histopathology in conjunction with *in situ* hybridization with specific probe is useful (Way et al., 2004).

4.3.3.3. Immunoassays

ELISA for detecting anti-KHV carp antibody is available for screening exposed fish (Ronen et al., 2003).

5. Control Methods

As with other viral infections, no treatment is possible. In addition, while research on a vaccine is on-going (Ronen et al., 2003), no commercial vaccine is, as yet, available.

For facilities that are affected or are suspected of being affected, the following control measures, where practical, are recommended:

- Restrictions on movements and transportation of fish
- Sanitary slaughtering of affected and suspected fish
- To eliminate contaminated pond water as a source of infection, treat water with chlorine prior to draining (OIE, 2003)
- Restriction of water movements onto and off farms
- For quarantine measures applied to movement of potential carriers see section 4.1.
- Due to the highly contagious nature of this disease, early detection is critical. Where practical, emergency harvest of food fish that remain fit for human consumption should be considered.
Selected references


Gilad, O; Yun, S; Andree, KB; Adkison, MA; Zlotkin, A; Bercovier, H; Eldar, A; Hedrick, RP. 2002. Initial characteristics of koi herpesvirus and development of a polymerase chain reaction assay to detect the virus in koi, Cyprinus carpio koi. Dis. Aquat. Org. 48: 101-108.


Tu, C; Weng, M-C; Shiau, J-R; Lin, S-Y. 2004. Detection of koi herpesvirus in koi Cyprinus carpio in Taiwan. Fish Pathol. 39: 109-110.

Figures and Figure Legends

Figure 1. Examples of carp at the early stages of infection with KHV. A: Common carp with no marked signs of disease apart from a small degree of fin rot and slight redness of abdomen. B: Koi carp with skin haemorrhages, congested fin and sunken eye, diagnosed positive for KHV by PCR.

Figure 2. Examples of common carp showing severe signs of KHV infection. A: Fish from an outbreak in Japan, demonstrating pale and necrotic gills, and sunken eye; B: Fish from an outbreak in West Java, demonstrating severely necrotic gills with possible secondary bacterial infection.
Figure 3. Section of gill tissue from KHV-infected carp. Note the hyperplasia and fusion of secondary lamellae.
Experts outside the region

<table>
<thead>
<tr>
<th>Expert</th>
<th>Laboratory</th>
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Experts in the region

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