**USA COMMENTS IN RED FONT**

CHAPTER 2.3.6.

**infection with koi herpesvirus**

**1. Scope**

Infection with koi herpesvirus (KHV) means infection with all genotypes of the pathogenic agent cyprinid herpesvirus-3 (CyHV-3), of the Genus *Cyprinivirus* in the Family *Alloherpesviridae* (Engelsma *et al.,* 2013; Haramoto *et al*., 2007; Waltzek *et al.,* 2009). ~~However, for familiarity, the abbreviation KHV will be used in this chapter.~~

**RATIONALE:** The pathogen name for which KHV is the acronym is already stated in the first sentence.

**2. Disease information**

**2.1. Agent factors**

**2.1.1. Aetiological agent**

KHV, also known as carp interstitial nephritis and gill necrosis virus (CNGV) (Ilouze *et al.,* 2010), has been classified as cyprinid herpesvirus-3 (CyHV-3) following the nomenclature of other cyprinid herpesviruses: CyHV-1 (carp pox virus, fish papilloma virus) and CyHV-2 (goldfish haematopoietic necrosis virus). Analysis of the complete genome has shown that CyHV-3 is closely related to CyHV-1, CyHV-2, anguillid herpesvirus-1 (AngHV-1) and distantly related to channel catfish virus (Ictalurid herpesvirus: IcHV-1) and Ranid (frog) herpesvirus (RaHV-1) (Waltzek *et al.,* 2005). CyHV-3 was designated the type species of the new *Cyprinivirus* genus within the *Alloherpesviridae* family, that also contains CyHV-1 and CyHV-2. However, the designation KHV has been retained in the *Aquatic Code* and *Aquatic Manual* for reasons of continuity and is used here synonymously with CyHV-3.

Early estimates of the genome size of KHV varied from at least 150 kbp to 277 kbp; the size is now confirmed as 295 kbp. Virus nucleocapsids have been measured at 100–110 nm in diameter and are surrounded by an envelope (review: Ilouze *et al.,* 2010). The enveloped virions range in size from 170 to 230 nm in the different infected cell types (Hedrick *et al.,* 2000; Miwa *et al.,* 2007;Miyazaki *et al,* 2008). Aoki *et al.* (2007) initially described the complete genome sequence of three isolates of ~~CyHV-3~~ KHV and the genome includes 164 open reading frames (ORFs) as well as 156 unique protein-coding genes. They suggested that the finding that 15 KHV genes are homologous with genes in IcHV-1 confirms the proposed place of KHV in the family Herpesviridae. Forty viral proteins and 18 cellular proteins are incorporated into mature virions.

**2.1.2. Survival and stability in processed or stored samples**

No information available.

**2.1.3. Survival and stability outside the host**

Studies in Israel have shown that KHV remains viable in water for at least 4 hours, but less than 21 hours, at water temperatures of 23–25°C (Perelberg *et al.,* 2003). Studies in Japan have shown a significant reduction in the infectious titre of KHV within 3 days in river or pond water or sediment samples at 15°C. However, KHV remained infective for >7 days when kept in environmental water samples that had been sterilised by autoclaving or filtration (Shimizu *et al.,* 2006).

**2.2. Host factors**

**2.2.1. Susceptible host species**

Species that fulfil the criteria for listing as susceptible to infection with KHV according to Chapter 1.5 of *Aquatic Animal Health Code* (*Aquatic Code*) are: all varieties and subspecies of common carp (*Cyprinus carpio*), and common carp~~/goldfish~~ hybrids (e.g. *Cyprinus carpio* × *Carassius auratus, Cyprinus carpio* × *Carassius carassius*).

**2.2.2. Species with incomplete evidence for susceptibility**

Species for which there is insufficient evidence to fulfil the criteria for listing as susceptible to infection with KHV according to Chapter 1.5 of the *Aquatic Code* are: Goldfish (*Carassius auratus*), grass carp (*Ctenopharyngodon idella*) and Crucian carp (*Carassius carassius*).

In addition, pathogen-specific positive polymerase chain reaction (PCR) and or *in-situ* hybridisation results have been reported in the following organisms, but an active infection has not been demonstrated:

| **Family** | **Scientific name** | **Common name** |
| --- | --- | --- |
| Acipenseridae | *Acipenser gueldenstaedtii* | Atlantic sturgeon |
| *Acipenser ruthenus* × *Huso huso* | hybrid sterlet × beluga |
| *Acipencer oxyrinchus* | Russian sturgeon |
| Cyprinidae | *Leuciscus idus* | blue back ide |
| *Rutilus rutilus* | common roach |
| *Tinca tinca* | tench |
| *Hypophthalmichthys molitrix* | silver carp |
| Gammaridae | *Gammarus pulex* | scud (crustacean) |
| Nemacheilidae | *Barbatula barbatula* | stone loach |
| Percidae | *Gymnocephalus cernuus* | Eurasi~~e~~an~~s~~ ruffe |
| *Perca fluviatilis* | European perch |
| ~~Salmonidae~~ | *~~Oncorhynchus mykiss~~* | ~~rainbow trout~~ |
| Unionidae | *Anodonta cygnea* | swan mussel |

**2.2.3. ~~Non-susceptible species~~**

~~Species that have been found non-susceptible to infection with KHV according to Chapter 1.5. of the~~ *~~Aquatic Code~~* ~~are:~~

| **~~Family~~** | **~~Scientific name~~** | **~~Common name~~** |
| --- | --- | --- |
| ~~Agamidae~~ | *~~Intellagama~~**~~lesueurii~~* | ~~Eastern water dragon~~ |
| ~~Ambassidae~~ | *~~Ambassis agassizii~~* | ~~olive perchlet~~ |
| ~~Anguillidae~~ | *~~Anguilla australis~~* | ~~short-finned eel~~ |
| ~~Ariidae~~ | *~~Neoarius graeffei~~* | ~~salmon catfish~~ |
| ~~Chelidae~~ | *~~Emydura macquarii~~* | ~~Macquarie short-necked turtle~~ |
| ~~Clupeidae~~ | *~~Nematalosa erebi~~* | ~~bony bream~~ |
| ~~Eleotridae~~ | *~~Hypseleotris~~* ~~sp.~~ | ~~carp gudgeon~~ |
| ~~Galaxiidae~~ | *~~Galaxias maculatus~~* | ~~common galaxias~~ |
| ~~Limnodynastidae~~ | *~~Limnodynastes tasmaniensis~~* | ~~spotted marsh frogs~~ |
| ~~Melanotaeniidae~~ | *~~Melanotaenia duboulayi~~* | ~~crimson-spotted rainbowfish~~ |
| ~~Mordaciidae~~ | *~~Mordacia mordax~~* | ~~short-headed lamprey ammocoetes~~ |
| ~~Mugilidae~~ | *~~Mugil cephalus~~* | ~~sea mullet~~ |
| ~~Parastacidae~~ | *~~Cherax destructor~~* | ~~common yabby~~ |
| ~~Pelodryadidae~~ | *~~Litoria~~**~~peronii~~* | ~~Peron’s tree frog~~ |
| ~~Percichthyidae~~ | *~~Maccullochella peelii~~* | ~~Murray cod~~ |
| *~~Macquaria ambigua~~* | ~~golden perch~~ |
| ~~Plotosidae~~ | *~~Tandanus tandanus~~* | ~~eel-tailed catfish~~ |
| ~~Retropinna~~ | *~~Retropinna semoni~~* | ~~Australian smelt~~ |
| ~~Terapontidae~~ | *~~Bidyanus bidyanus~~* | ~~silver perch~~ |

**2.2.~~4~~ 3. Likelihood of infection by species, host life stage, population or sub-populations**

For the purposes of Table 4.1, larvae and fry up to approximately 1 g in weight may be considered to be early life stages, fingerlings and grower fish up to 250 g may be considered to be juveniles, and fish above 250 g may be considered to be adults.

All age groups of fish, from juveniles upwards, appear to be susceptible to infection with KHV but, under experimental conditions, 2.5–6 g fish were more susceptible than 230 g fish (Perelberg *et al.,* 2003). Carp larvae appear to be tolerant to infection with KHV.

Common carp or varieties, such as koi or ghost (koi × common) carp, are most susceptible and should be preferentially selected for virus detection, followed by any common carp hybrids, such as goldfish × common carp or crucian carp × common carp. Experimental challenges studies by Ito *et al.,* 2014a; 2014b, demonstrated that mortality due to infection with KHV was higher in indigenous Japanese carp (95–100%) compare with domesticated common carp and koi carp, where mortality varied from 30% to 95% and from 35% to 100%, respectively.

**2.2.~~5~~ 4. Distribution of the pathogen in the host**

Gill, kidney, gut and spleen are the organs in which KHV is most abundant during the course of clinical disease (Gilad *et al.,* 2004). In fish surviving experiment challenge by immersion, KHV DNA was more likely to be detected from the caudal fin and brain compared with gill and kidney (Ito *et al*., 2014b).

**2.2.~~6~~ 5. Aquatic animal reservoirs of infection**

There is evidence to indicate that survivors of infection with KHV may become persistently infected with virus and may retain the virus for long periods without expression of clinical signs of infection. The virus has been shown to persist in common carp experimentally infected at a permissive temperature and subsequently maintained at a lower than permissive temperature (St-Hilaire *et al.,* 2005). Researchers in Japan conducted a PCR and serological survey of ~~CyHV-3~~ KHV in Lake Biwa in 2006, where episodic outbreaks of infection with KHV had been reported in the 2 years following a major outbreak in 2004. Further analysis of the surviving population showed that 54% of the older carp were seropositive and 31% PCR positive. The maintenance of high levels of antibody to the virus suggests that latent virus may be reactivating periodically in some animals, leading to excretion and a low level of virus circulation in the population, which boosts herd immunity.

**2.2.~~7~~ 6. Vectors**

No species of vector have been demonstrated to transmit KHV to susceptible species. Studies in Japan have however, reported the detection of ~~CyHV-3~~ KHV DNA in plankton samples and, in particular, Rotifera species ~~Plankton samples were collected in 2008 from Iba-naiko, a shallow lagoon connected to Lake Biwa, a favoured carp spawning area~~ (Minamoto *et al*., 2011). ~~Statistical analysis revealed a significant positive correlation between CyHV-3 in plankton and the numbers of Rotifera and the authors suggested that CyHV-3 binds to or is concentrated by the filter feeding behaviour of Rotifera species. In an earlier report of a study in Poland, CyHV-3~~ KHV ~~was~~ has also been detected by PCR in swan mussels (*Anodonta cygnea*) and freshwater shrimp (*Gammarus pulex*) (Kielpinski *et al*., 2010). ~~The invertebrates were collected from ponds in Southern Poland where outbreaks had occurred in common carp populations over 5 to 6 years. More work is needed to determine how long the infectious virus persists and remains viable in the invertebrates in the absence of the host species.~~

**2.3. Disease pattern**

**2.3.1. Mortality, morbidity and prevalence**

The clinical signs of infection may become apparent 3–21 days after naïve fish have been introduced to a pond containing infected fish (Bretzinger *et al.,* 1999; Hedrick *et al*., 2000). Morbidity of affected populations can be 100%, and mortality 70–100% (Bretzinger *et al.,* 1999; Haenen *et al.,* 2004). However, in several experiments, differential resistance to infection with KHV among common carp strains was reported (Dixon *et al.*, 2009; Ito *et al*., 2014a; Shapira *et al*., 2005). In these reports, the cumulative mortalities of the most resistant strains were approximately 40%. Secondary and concomitant bacterial or parasitic infections are commonly seen in diseased carp and may affect both the mortality rate and clinical signs of infection (Haenen *et al.,* 2004).

**2.3.2. Clinical signs, including behavioural changes**

During an outbreak of infection with KHV there will be a noticeable increase in mortality in the population. All age groups of fish, except larvae, appear to be susceptible to infection with KHV, although, under experimental infection, younger fish (up to 1 year of age) are more susceptible to infection. Changes to the skin are also commonly observed and include: focal or total loss of epidermis, irregular patches of pale colouration or reddening, excessive or reduced mucous secretion (on skin or gills) and sandpaper-like skin texture. Other clinical signs include enophthalmia (sunken eyes) and haemorrhages on the skin and base of the fins, and fin erosion.

Fish become lethargic, separate from the shoal and gather at the water inlet or sides of a pond and gasp at the surface of the water. Some fish may experience loss of equilibrium and disorientation, but others may show signs of hyperactivity.

**2.3.3. Gross pathology**

There are no pathognomic gross lesions. However, the most consistent gross pathology is seen in the gills, which can vary in extent from pale necrotic patches to extensive discolouration, severe necrosis and inflammation. Internal lesions are variable in occurrence and often absent in cases of sudden mortality. Other gross pathologies that have been reported include adhesions in the abdominal cavity, with or without abnormal colouration of internal organs (lighter or darker). The kidney or liver may be enlarged, and they may also exhibit petechial haemorrhages. Co-infections, for example with ectoparasites such as gill monogeneans, may alter the observed gross pathology.

**2.3.4. Modes of transmission and life cycle**

Virus is shed via faeces, urine, gills and skin and the main mode of transmission of KHV is horizontal. Early reports suggested that the gills are the major portal of virus entry in carp (Dishon *et al.,* 2005; Gilad *et al.,* 2004; Pikarsky *et al*., 2004).

However, a more recent experimental study has demonstrated that the skin covering the fins and body of the carp is the major portal of entry for KHV (Costes *et al.,* 2009). Another study has shown that KHV DNA was detected in two of three fish from the caudal fin and gill, and caudal fin and spleen one day after exposure to sub-clinically infected fish (Ito *et al*., 2014a; 2014b). The virus spreads systemically from main points of entry to the internal organs; high levels of KHV DNA have been detected in kidney, spleen, liver and gut tissue (Dishon *et al.,* 2005; Pikarsky *et al*., 2004). The assembly and morphogenesis of KHV in infected cells is the same as other herpesviruses (Miwa *et al.*, 2007). An ultrastructural examination of experimentally infected carp has provided evidence for immature capsids and mature nucleocapsid assembly in the nucleus and further maturation of the virion in the cytoplasm of infected cells. Hyper-secretion of mucous is very evident in the early stages of infection with KHV and KHV DNA has been detected at high levels in mucous sampled from experimentally infected carp (Gilad *et al.,* 2004). This is further evidence for active involvement of the skin in viral pathogenesis and an important site of virus shedding. Excretion of virus via urine and faeces may also be an important mechanism for virus shedding; infectious virus has been detected in faeces sampled from infected carp (Dishon *et al.,* 2005; Gilad *et al*., 2004).

**2.3.5. Environmental factors**

Disease patterns are influenced by water temperature, virulence of the virus, age, population genetics and condition of the fish, population density and stress factors (e.g. transportation, spawning, poor water quality). The disease is temperature dependent, occurring mainly between 16 and 29°C (Haenen *et al.,* 2004; Hedrick *et al*., 2000; Perelberg *et al.,* 2003; Sano *et al.,* 2004). Under experimental conditions, infectious virus was continually shed for a longer period from infected common carp at 16°C than those kept at 23°C or 28°C (Yuasa *et al.,* 2008). However, experimental challenge resulted in high mortality at 28°C but not at 29°C or 30°C, nor at 13°C (Gilad *et al.,* 2004; Ilouze *et al.,* 2010) (optimal temperature range for viral replication may vary with the virus strain).

**2.3.6. Geographical distribution**

Following the first reports of infection with KHV in Israel and Germany in 1998 and detection of KHV DNA in tissue samples taken during a mass mortality of carp in the UK in 1996, the geographical range of the disease has become extensive and includes most continents, including Europe, Asia, the Middle East, Southern Africa, and North America.

See WAHIS (<https://wahis.oie.int/#/home>) for recent information on distribution at the country level.

**2.4. Biosecurity and disease control strategies**

**2.4.1. Vaccination**

A safe and effective commercial vaccine is not currently widely available. However, live attenuated virus has been used to vaccinate carp. The vaccine preparation induced antibody against the virus and the duration of the protection was at least 8 months (Ilouze *et al.,* 2010). The vaccine was licensed for emergency use in Israel and has been widely used in carp farms across the country. Various vaccine candidates against KHV have been developed. Results of studies in Japan have shown that oral administration of a liposome-based vaccine containing inactivated KHV was also effective in protecting carp against clinical disease (reviewed by Ilouze *et al.,* 2010). A vaccine candidate based on the double deletion of ORF56 and ORF57 was produced using BAC cloning technology, and the effectiveness of attenuated recombinant vaccines has been demonstrated in experimental challenge experiments (Boutier *et al.*, 2015). The DNA vaccines consisting of plasmids encoding ORF25, ORF81 and ORF 149 showed efficient results under lab conditions (Hu *et al.,* 2020; Zhou *et al.,* 2014a; 2014b;).

**2.4.2. Chemotherapy including blocking agents**

Chemotherapy is not currently available, however, the antiviral activity of exopolysaccharides against KHV *in vitro* has been reported (Reichert *et al.*, 2017).

**2.4.3. Immunostimulation**

There is currently no published information on the use of immunostimulants to control infection with KHV in carp. However, it is known to be an area of research interest (Reichert *et al.,* 2017).

**2.4.4. Breeding resistant strains**

Differential resistance to infection with KHV, but not to virus entry, has been shown among different carp strains (Dixon *et al.,* 2009; Ito *et al*., 2014a; 2014b; Shapira *et al.,* 2005). The progeny of crosses of two strains of domesticated carp and one strain of wild carp were challenged by experimental or natural infection. The lowest survival rate was approximately 8% but the survival rate of the most resistant strain was 60.7% for experimental exposure and 63.5% for natural exposure in ponds (Shapira *et al.,* 2005). In a more recent resistance study, 96 families derived from di-allele crossing of four European/Asian strains of common carp were experimentally challenged with KHV. Survival rates of the five most resistant crosses in the final virus challenge trial ranged from 42.9 to 53.4% (Dixon *et al.,* 2009).

**2.4.5. Inactivation methods**

The virus is inactivated by UV radiation at a dose of 4.0 × 103 μ Ws/cm2, temperatures above 50°C for 1 minute and by iodophor (200 mg litre–1) treatment for 30 seconds at 15°C ~~(Kasai~~ *~~et al~~*~~., 2005)~~. The following disinfectants are also effective for inactivation: iodophor at 200 mg litre–1 for 20 minutes, benzalkonium chloride at 60 mg litre–1 for 20 minutes, ethyl alcohol at 30% for 20 minutes and sodium hypochlorite at 200 mg litre–1 for 30 seconds, all at 15°C (Kasai *et al.,* 2005).

**2.4.6. Disinfection of eggs and larvae**

Disinfection of the surface of the eggs can be achieved by iodophor treatment (Kasai *et al.,* 2005). There are no publications on the disinfection of larvae.

**2.4.7. General husbandry**

Biosecurity measures should include ensuring that new introductions of fish are from disease-free sources and installation of a quarantine system where new fish can be held with sentinel fish at permissive temperatures for infection with KHV. The fish should be quarantined for a minimum of 4 weeks to 2 months before transfer to the main site and mixing with naïve fish. Hygiene measures on site should include disinfection of eggs, regular disinfection of ponds, chemical disinfection of farm equipment, careful handling of fish to avoid stress and safe disposal of dead fish.

**3. Specimen selection, sample collection, transportation and handling**

**3.1. Selection of populations and individual specimens**

Clinical inspections should be carried out during a period when the water temperature is ~~conducive to development of clinical disease, i.e.~~ conducive to development of clinical disease, i.e. All production units (ponds, tanks, net-cages, etc.) should be inspected for the presence of dead, weak or abnormally behaving fish. If moribund fish or fish showing clinical signs are sampled, the probability of detecting KHV is higher than if randomly selected, apparently healthy fish are sampled.

**RATIONALE:** Corrected font size.

~~Fish to be sampled are selected as follows:~~ For the purposes of disease surveillance, fish to be sampled are selected as follows:

i) ~~Susceptible species should be sampled proportionally or following~~ The most susceptible species should be sampled preferentially (see Section 2.2.3). Other susceptible species listed in Section 2.2.1 should be sampled proportionally.

ii) Risk-based criteria ~~for targeted selection of~~ should be employed to preferentially sample lots or populations with a history of abnormal mortality or potential exposure events (e.g. via untreated surface water, wild harvest or replacement with stocks of unknown disease status) or where there is evidence of poor water quality or husbandry. Younger fish up to 1 year are more susceptible to clinical disease and are recommended for sampling. If more than one water source is used for fish production, fish from all water sources should be included in the sample.