[5] Diseases Caused By Viral Pathogens

international trade of aquatic animals.

1. FRESHWATER FISH

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1.1. Synopsis/Abstract

This section describes the viral diseases affecting warm-water fin-fish in fresh water. Although many viral diseases and the causative viruses have been reported to date, this section focuses on the five most important viral diseases.

1.2. Introduction

Fresh water fish form the largest segment of the world aquaculture production and a major part of the catch-fish industry in inland waters. The harvest of farmed warm-water fish is increasing, and consequently, fry production of fish species for aquaculture has also rapidly increased and disease problems have become the largest obstacle in aquaculture operations. Some diseases of cultured fish (e.g. koi herpesvirus disease) have spread to wild populations of fish which pose serious threats to the ecosystem. .

Wolf (1988) described 59 fish viruses, and, subsequently, the number of fish viruses reported continues to increase so far. Viruses of warm-fresh water fish of major importance are shown in Table 1.1 (Sano et al, 2011). This section focuses on diseases caused by 5 of the viruses in the table: spring viremia of carp, channel catfish virus disease, koi herpesvirus disease, herpesviral hematopoietic necrosis and epizootic hematopoietic necrosis. These diseases are transboundary aquatic animal diseases, of international concern due to their significance in the international trade of aquatic animals. Most of the diseases in this section are currently listed by the World Organisation for Animal Health (OIE) (Aquatic Animal Health Code 2012).

Virus	Taxonomy (Family)	Main Host
DNA Virus		
Carp herpesvirus (CHV) (=CyHV-1)	Alloherpesviridae	Carp (*Cyprinus carpio)*
Goldfish hematopietic necrosis virus (GFHNV) (=CyHV-2)	Alloherpesviridae	Goldfish (*Carassius auratus*)
Koi herpesvirus (KHV) (=CyHV-3**)**	Alloherpesviridae	Carp
Channel catfish virus (CCV) (=IcHV-1)	Alloherpesviridae	Channel catfish (*Ictalurus punctatus*)
Ictalurus melas herpesvirus (IcmHV) (=IcHV-2)	Alloherpesviridae	Black bullhead (*Ameiurus melas*)
Herpesvirus anguillae (HVA) (AngHV-1)	Alloherpesviridae	Japanese eel (*Anguilla japonica*), European
White sturgeon herpesvirus 1 (WSHV-1) (=AciHV-1)	Alloherpesviridae	White sturgeon (*Acipenser transmontanus*)
White sturgeon herpesvirus 2 (WSHV-2) (=AciHV-2)	Alloherpesviridae	White sturgeon

Epizootic hematopoietic necrosis virus (EHNV)	Iridoviridae	Redfin perch (*Perca fluviatilis*)
European catfish virus (ECV)	Iridoviridae	Black bullhead
European sheatfish virus (ESV)	Iridoviridae	Sheatfish (*Silurus glanis*)
Largemouth bass virus (LMBV)	Iridoviridae	Largemouth bass (*Micropterus salmoides*)
White sturgeon iridovirus (WSIV)	Iridoviridae	White sturgeon
Infectious spleen and kidney necrosis virus (ISKNV)	Iridoviridae	Various freshwater species
Carp edema virus (CEV)	Pox-like virus	Carp
RNA Virus		
Spring viremia of carp virus (SVCV)	Rhabdoviridae	Carp
Viral hemorrhagic septicaemia virus (VHSV) (genogroup IVb)	Rhabdoviridae	Various freshwater species
Pike fry rhabdovirus (PFRV)	Rhabdoviridae	Northern pike (*Esox lucius*)
Perch rhabdovirus (PRV)	Rhabdoviridae	Redfin perch
Snakehead rhabdovirus (SHRV)	Rhabdoviridae	Snakehead fish (*Ophicephalus striatu*)
Aquabirnaviruses (e.g. Eel virus European)	Birnaviridae	Various freshwater species
Golden shiner virus (GSV)	Reoviridae	Golden shiner (*Notemigonus crysoleucas*)
Grass carp reovirus (GCRV)	Reoviridae	Grass carp (*Ctenopharyngodon idella*)

Table 1.1. Major viruses of warm water freshwater species.

1.3. Spring Viremia of Carp

1.3.1. Introduction

Spring viremia of carp (SVC) is an acute, systemic, contagious disease caused by a rhabdovirus. SVC in carp typically occurs at 11-17°C, predominantly in spring. Mortality can reach to 30-70 %. This disease is currently listed by the OIE. Selected references or reviews: (Wolf, 1988; Sano et al, 2011; Aquatic Animal Health Code 2012; Fijan et al, 1971; Ahne et al, 2002; Walker and Winton, 2010; OIE, 2013; Stone et al, 2003).

1.3.2. Disease Agent

Virus: spring viremia of carp virus (SVCV) (available collection: ATCC VR-1390)
Virus taxonomy: genus *Vesiculovirus*, family Rhabdoviridae, order Mononegavirales
Morphology: typical bullet shaped virion with 60-90 nm wide and 80-180 nm long
Virion proteins: 5 structural proteins: L (238 kDa), G (57 kDa), N (47 kDa), P (35 kDa) and M (25 kDa)
Genome: single-stranded negative-sense RNA of ca. 11,000 nucleotide bases, encoding the structural proteins in the order 3'- N-P-M-G-L-5'; complete genome sequence: accession No. U18101, AJ318079, DQ097384, DQ491000, EU177782

Serotype: single (SVCV and pike fry rhabdovirus (PFRV) can be two serotypes of a single virus species)
Genotype: single genotype (Genogroup I) with 4 subgenogroups (Ia to Id) consisted with the geographical origin

1.3.3. Geographical Distribution

European countries, USA, Canada and China

1.3.4. Host Range

Carp (*Cyprinus carpio*) and other cyprinid species (eg. goldfish (*Carassius auratus*), grass carp (*Ctenopharyngodon idella*), crucian carp (*Carassius carassius*)).

1.3.5. Diagnostic Methods

Clinical signs: External signs are non-specific, but likely include skin darkening, abdominal distension, exophthalmos, petechial hemorrhage in the skin and gills, and pale gills.

Gross pathology: Internal signs are dominated by edema in all organs, hemorrhage, peritonitis and catarrhal enteritis. Excess ascites may be bloody. Petechia is evident in the muscles and internal organs including swim-bladder.

Histopathology: Changes including hemorrhage, hyperemia, multiple focal necrosis, perivascular inflammation, and edema and necrosis of blood vessels can be observed in all major organs, especially liver, kidney and spleen.

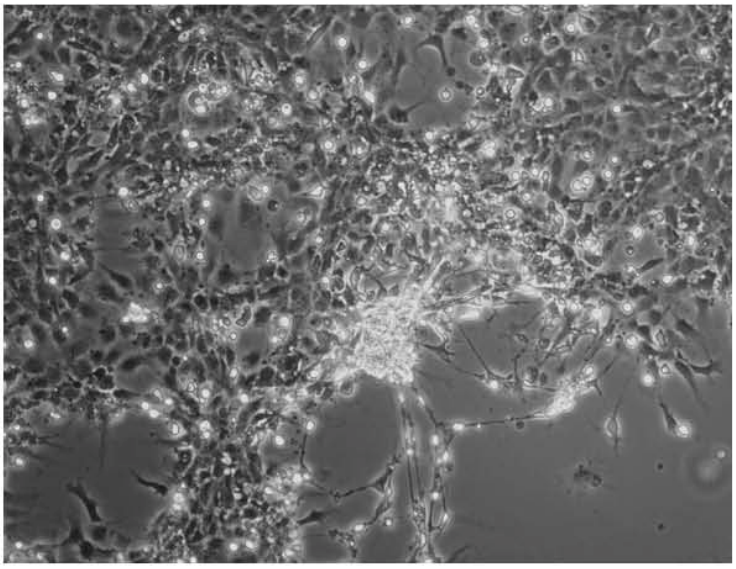

Figure 1.1. CPE on EPC cells following infection with SVCV.

Diagnosis: Specific diagnosis is generally based on the isolation of SVCV in cell culture (EPC or FHM) at 20°C by inoculation with homogenates of kidney, spleen, liver and

encephalon, followed by the identification. The typical CPE is of the rounded cells [Figure 1.1]. Isolated virus is identified using serological techniques (virus neutralization, IFAT, ELISA) and nucleotide based methods (RT-PCR, real-time RT-PCR or LAMP).

1.3.6. Control

SVCV is generally transmitted horizontally. Outbreaks can be prevented or stopped by raising water temperatures above 20°C. No chemotherapeutic treatments and commercial vaccines are currently available. General biosecurity measures and regular hygiene practices on farm level are applicable. Avoidance of crowding during winter and early spring is essential to reduce spread of the virus. A fish selection program resulted in high resistance of the Krasnodar strain of carp.

1.4. Channel Catfish Virus Disease

1.4.1. Introduction

Channel catfish virus disease (CCVD) is an acute, systemic, contiguous and highly species-specific disease of young channel catfish in the USA caused by an alloherpesvirus. CCVD in channel catfish typically occurs at high water temperature ranging approximately 20-30°C. Mortality can occasionally approach 100%. Selected references or reviews: (Wolf, 1988; Sano et al, 2011; Wolf and Darlington, 1971; Plumb, 1989 ; Davison, 1992 ; Camus, 2004 ; Hanson et al, 2011 ; Waltzek et al, 2009)

1.4.2. Disease Agent

Virus: Channel catfish virus (CCV) (available collection: ATCC VR-665)
Virus taxonomy: genus *Ictalurivirus*, family Alloherpesviridae, order Herpesvirales
Morphology: 175-200 nm virion consisted of 100 nm icosahedral nucleocapsid with an envelop
Virion proteins: 32 polypeptides detected
Genome: double-stranded DNA of 134 kbp with 90 genes predicted; complete genome sequence: accession No. NC_001493
Serotype: single
Genotype: not available

1.4.3. Geographical Distribution

USA, Mexico

1.4.4. Host Range

Channel catfish (*Ictalurus punctatus*) and blue catfish (*Ictalurus furcatus*). White catfish (*Ictalurus cams*) is susceptible to experimental infection.

1.4.5. Diagnostic Methods

Clinical signs: Signs can vary and depend on the degree of kidney damage due to CCV

multiplication, and usually include distension of abdomen, exophthalmia, swollen and protruding vent, hemorrhage at the base of ventral and caudal fins, in gills and skin.

Gross pathology: The peritoneal cavity is hyperemic and contains a clear, yellowish or slightly reddish fluid. Liver and kidney may be pale, with or without hemorrhage or petechiae. The spleen is congested and dark. A yellowish mucoid material is present in the intestine.

Histopathology: Severe changes, consisting of edema, hemorrhage and necrosis can be observed in the internal organs, especially in kidney. The hematopoietic tissue shows an increase in lymphoid cells, edema, necrosis and accumulation of macrophages. Necrosis and occasional hemorrhage develop in nephrons.

Diagnosis: Specific diagnosis is generally based on the isolation of CCV in cell culture (CCO and BB) at 25°C by inoculation with homogenates of kidney and spleen, followed by the identification. The typical CPE is of cell enlargement and syncytium formation. Isolated virus is identified using serological techniques (virus neutralization, IFAT, ELISA) and also nucleotide based methods (PCR, real-time RT-PCR). PCR is at present, the most useful method.

1.4.6. Control

CCV is generally transmitted horizontally. The reduction of water temperature to 19°C or lower may decrease the mortality. No chemotherapeutic treatments and commercial vaccines are currently available. General biosecurity measures and regular hygiene practices on farm level are applicable. Lower stocking densities for production of fingerlings and appropriate daily feeding rates are important, especially during high water temperatures. Fingerlings should be harvested and handled only below 20°C. Breeding for resistance and hybridization of channel catfish strains is a promising approach.

1.5. Koi Herpesvirus Disease

1.5.1. Introduction

Koi herpesvirus disease (KHVD) is an acute, systemic, highly contagious disease caused by an alloherpesvirus. KHVD typically occurs at 17-28°C. Mortality can approach 100%. This disease is currently listed by the OIE. Selected references or reviews: (Wolf, 1988; Sano et al, 2011; Aquatic Animal Health Code 2012; OIE, 2013; Hanson et al, 2011; Waltzek et al, 2009; Hedrick et al, 2000; Haenen et al, 2004; Haenen and Hedrick, 2006; Michel et al, 2010 ; Kurita et al, 2009)[1-3, 7, 13-19].

1.5.2. Disease Agent

Virus: koi herpesvirus (KHV) (=cyprinid herpesvirus 3(CyHV-3)) (available collection: ATCC VR-1592)
Virus taxonomy: genus *Cyprinivirus*, family Alloherpesviridae, order Herpesvirales
Morphology: 170-200 nm virion consisted of 110-120 nm icosahedral nucleocapsid with an envelop

Virion proteins: 40 polypeptides detected
Genome: double-stranded DNA of 295 kbp with 163 genes predicted; complete genome sequence: accession No. NC_009127 (DQ177346 (strain I); DQ657948 (strain U); AP008984 (strain J=TUMST1)
Serotype: single
Genotype: two distinct (European and Asian) lineages

1.5.3. Geographical Distribution

European countries, Asian countries, North America, Israel, South Africa

1.5.4. Host Range

Carp (*Cyprinus carpio*) (including ornamental varieties such as koi)

1.5.5. Diagnostic Methods

Clinical signs: The most consistent sign is an irregular discoloration of the gills consistent with necrosis (Figure 1.2). Other signs include anorexia, exophthalmia, fin erosion, hemorrhage on the skin and base of the fins, pale irregular patches on the skin associated with excess mucus secretion.

Figure 1.2. A common carp with KHVD showing necrosis of the gill filaments and enophthalmia.

Gross pathology: Internal gross signs are inconsistent, but enlarged kidney, swollen spleen and heart are occasionally observed.

Histopathology: Changes are not consistent, but necrosis of gill tissues and hyperplasia and hypertrophy of the branchial epitherial cells and fusion of adjacent secondary lamellae are commonly found (Figure 1.3). Inflammation, necrosis, and nuclear swelling, margination of chromatin and plae diffuse eosinophilic inclusions may be observed in the organs including gill, kidney, gastrointestinal system and skin.

Diagnosis: Specific diagnosis is based on direct method such as virus isolation, viral antigen detection using IFAT or ELISA, and viral DNA amplification assay using PCR, real-time PCR or LAMP. PCR is currently considered most reliable method. Virus isolation can be done in KF-1 or CCB at 20°C, but is probably difficult to achieve reliably. The typical CPE is of syncytium formation and intense cytoplasmic vacuolation (Figure 1.4). Antibody-capture ELISA is helpful for screening the fish experienced with the disease.

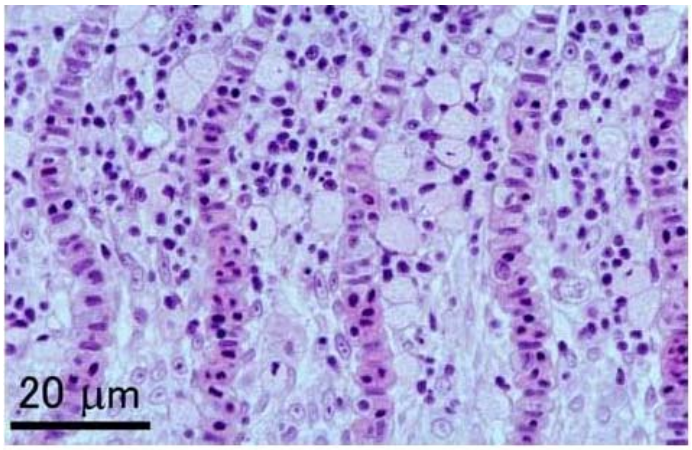

Figure 1.3. Tissue section of the gills of common carp infected with KHV showing fusion of secondary lammelae. Courtesy of Dr. S. Miwa.

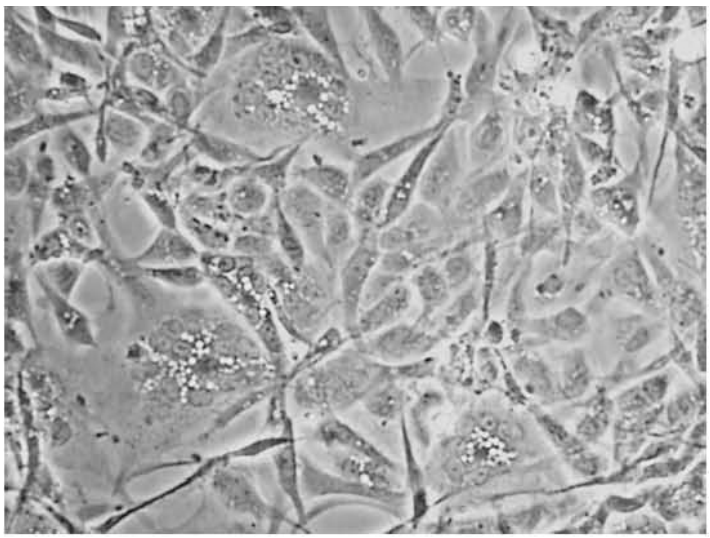

Figure 1.4. CPE on CCB cells following infection with KHV.

1.5.6. Control

KHVD is generally transmitted horizontally. Outbreaks can be prevented or stopped by raising water temperatures above 28°C. No chemotherapeutic treatments are available. A vaccine using attenuated virus is commercially licensed in Israel. Survivor fish in KHVD, which is persistently or latently infected with the virus, is considered a potential risk as an

infection source through worldwide trade, and, therefore, koi farm should aim to produce KHV-free fish. Egg or fish should be introduced in aquaculture facility from KHV-free farm. Breeding for resistance of carp strains is a promising approach.

1.6. Herpesviral Hematopoietic Necrosis

1.6.1. Introduction

Herpesviral hematopoietic necrosis (HVHN) is an acute, systemic, highly contagious disease caused by an alloherpesvirus. HVHN in goldfish typically occurs at 15-25°C. Mortality can reach 100%. Selected references: (Waltzek et al, 2009; Jung and Miyazaki 1995; Waltzek et al, 2009; Goodwin et al, 2006; Li et al, 2003; Goodwin et al, 2009; Jeffery et al, 2007; Wang et al, 2012).

1.6.2. Disease Agent

Virus: goldfish hematopoietic necrosis virus (GFHNV) (=cyprinid herpesvirus 2(CyHV-2))
Virus taxonomy: genus *Cyprinivirus*, family Alloherpesviridae, order Herpesvirales
Morphology: 170-220 nm virion consisted of 115-117 nm icosahedral nucleocapsid with an envelop
Virion proteins: not available
Genome: double-stranded DNA
Serotype: not available
Genotype: not available

1.6.3. Geographical Distribution

Japan, Taiwan, China, USA, UK, Australia, Czech (possibly distributed by hobbyist aquariums worldwide)

1.6.4. Host Range

Goldfish (*Carassius auratus*) and Prussian carp (*Carassius gibelio*)

1.6.5. Diagnostic Methods

Clinical signs: Diseased fish show a pale skin and necrotic pale gills sometimes with white patches, and occasionally abdominal distension and exophthalmia.

Gross pathology: Pale gills and liver, ascites, splenomegaly with white nodular lesions, swollen pale kidney, and an intestine devoid of food are often observed.

Histopathology: The most severe lesions are found in the kidney and spleen. Necrosis in the hematopoietic cells and renal tubular epithelia and glomeruli in the kidney, and extensive necrosis of pulp and sheathed arteries are observed.

Diagnosis: Specific diagnosis is based on direct detection by PCR or real-time PCR.

GFHNV is difficult to isolate in cell culture and is typically lost after several passages in EPC, FHM and KF-1. Successful propagation using GFF (GF-1) derived from goldfish fin has been reported. The typical CPE is of intense cytoplasmic vacuolation similar with those of KHV.

1.6.5. Control

GFHNV is generally transmitted horizontally. Outbreaks in goldfish can be stopped by raising water temperatures at 33°C for over 4 days, and the treated fish may become carrier of the virus. No chemotherapeutic treatments and commercial vaccines are currently available. General biosecurity measures and regular hygiene practices on farm level are applicable. Susceptibility to the virus is different among goldfish varieties, and breeding for resistance of goldfish strains is a promising approach.

1.7. Epizootic Hematopoietic Necrosis

1.7.1. Introduction

Epizootic hematopoietic necrosis (EHN) is an acute, systemic, contagious disease in mostly redfin perch in Australia caused by an iridovirus. EHN in redfin perch occurs at 12-21°C. Mortality, especially in juvenile redfin perch, can approach 100%. This disease is currently listed by the OIE. Selected references or reviews: (Sano et al, 2011; Aquatic Animal Health Code 2012; OIE, 2013 ; Langdon et al, 1986 ; Chinchar, 2002 ; Chinchar et al, 2009; Whittington et al, 2010; Jancovich et al, 2010).

1.7.2. Disease Agent

Virus: Epizootic hematopoietic necrosis virus (EHNV)
Virus taxonomy: genus *ranavirus*, family Iridoviridae
Morphology: approximately 175 nm icosahedral virion with an inner lipid layer
Virion proteins: 30 polypeptides detected (Frog virus 3 (FV-3), a type strain of *Ranavirus*))
Genome: double-stranded DNA of 127 kbp with 100 genes predicted; complete genome sequence: accession No. FJ433873
Serotype: not available (EHNV and ictalurid isolates, European catfish virus (ECV) and European sheatfish virus (ESV), show serological and genetic relatedness to FV-3)
Genotype: not available

1.7.3. Geographical Distribution

Australia

1.7.4. Host Range

Redfin perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*). A number of other species such as Macquarie perch (*Macquaria australasica*), silver perch (*Bidyamus bidyanus*), mosquito fish (*Gambusia affinis*), mountain galaxias (*Galaxia olidus*) are susceptible to experimental infection.

1.7.5. Diagnostic Methods

Clinical signs: Signs are non-specific, but likely include skin darkening, erythema around the brain and nostrils, and hemorrhage around bases of fins.

Gross pathology: Internal gross signs are inconsistent, but some fish show enlargement of kidney, liver or spleen. There may be focal whitish lesions in the liver corresponding to areas of necrosis.

Histopathology: Changes consist of acute focal, multifocal or locally extensive coagulative or liquefactive necrosis of liver, hematopoietic kidney and spleen, and also necrosis in heart, pancreas, gastrointestinal tract, gill and pseudobranch.

Diagnosis: Specific diagnosis is generally based on the isolation of EHNV in cell culture (BF-2 or FHM) at 22°C by inoculation with homogenates of liver, kidney and spleen, followed by the identification. The typical CPE is of the rounded cells [Figure 1.5]. Isolated virus is identified using serological techniques (ELISA) and nucleotide based methods (PCR-restriction endonuclease analysis).

1.7.6. Control

EHNV is generally transmitted horizontally. Outbreaks may be prevented by shifting water temperatures below 12°C. No chemotherapeutic treatments and commercial vaccines are currently available. Since EHN is endemic in the limited water basin of Australia, general biosecurity measures are applicable for prevention of spreading this disease.

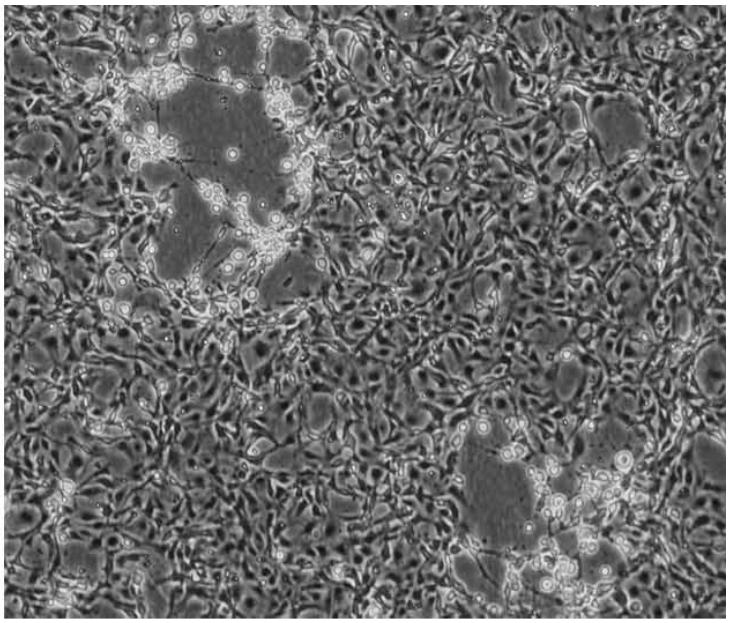

Figure 1.5. CPE on BF-2 cells following infection with EHNV.