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[5] Diseases Caused By Viral Pathogens

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2. Marine Fish

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2.1. Synopsis

With the rapid development of mass seed production techniques in fish, aquaculture targeting a variety of marine fish species has been intensively conducted in various areas of the world since 1980s. During fish rearing in hatcheries and farms, however, infectious diseases with high mortalities have frequently occurred, resulting in severe economical losses. It is no doubt that disease is one of the most important limiting factors for further development of marine aquaculture (Liao, 2009). This chapter describes viral diseases of marine fish excepting for salmonids, with particular reference to iridoviral disease caused by megalocytiviruses (Iridoviridae) and viral nervous necrosis caused by betanodaviruses (Nodaviridae), both of which are of great importance in world marine aquaculture due to their high geographical distribution, host range and virulence.

2.2. Introduction

Major viruses of non-salmonid marine fish causing diseases are listed in Table 2.1. Flounder herpesvirus (FHV), which is classified in *Alloherpesviridae*, causes often mass mortality in hatchery-reared larvae of Japanese flounder (*Paralichthys olivaceus*) in Japan (Iida et al, 1989, 2008). The disease is characterized by epidermal hyperplasia on the fins and skin with vacuolar degeneration of the Malpighian cells. Cell culture isolation and propagation of FHV have not succeeded. Another herpesvirus belonging to *Alloherpesviridae*, Pilchard herpesvirus (PHV), was found in the affected Australasian pilchard (*Sardinops sagax neopilchardus*) during a large-scale epizootic in Australian and New Zealand coastlines (Whittington et al, 1997; Hyatt et al, 1997). Significant lesions are confined to the gills where the virus is consistently present. Aquatic birnaviruses, which belong to the genus *Birnavirus* (*Birnaviridae*), are differentiated from infectious pancreatic necrosis virus (IPNV) causing disease in salmonid fish (Smail and Snow 2011). Specified clinical cases in non-salmonid marine fish species by aquatic birnaviruses are limited to yellowtail (*Seriola quinqueradiata*) (Sorimachi and Hara 1985) and some flatfish species such as turbot (*Psetta maxima*), Atlantic halibut (*Hippoglossus hippoglossus*) and Senegalese sole (*Solea senegalensis*) (Castric et al, 1987; Mortensen et al, 1990 ; Rodriguez Saint-Jean et al, 1997).

Virus	**Taxonomy Family / Genus**	**Main host fish**	**Reference**
Flounder herpesvirus (FHV)	*Alloherpesviridae*	Japanese flounder (*Paralichthys olivaceus*)	[2] [3]
Pilchard herpesvirus (PHV)	*Alloherpesviridae*	Pacific sardine (*Sardinops sagax*)	[4] [5]
Aquabirnaviruses Yellowtail ascites virus (YTAV) and unspecified viruses	*Birnaviridae* / *Aquabirnavirus*	Yellowtail (*Seriola quinqueradiata*) Turbot (*Psetta maxima*) Atlantic halibut (*Hippoglossus hippoglossus*)	[7] [8] [9][10]

		Senegalese sole (*Solea senegalensis*)	
Lymphocystis disease virus (LCDV)	*Iridoviridae* / *Lymphocystisvirus*	Various marine and freshwater species	[11]
Red sea bream iridovirus (RSIV) Infectious spleen and kidney necrosis virus (ISKNV)	*Iridoviridae* / *Megalocytivirus*	Various marine and brackishwater species	[20] [23] [29]
Betanodaviruses (NNV)	*Nodaviridae* / *Betanodavirus*	Various marine species	[54] [60]
Viral haemorrhagic septicaemia virus (VHSV)	*Rhabdoviridae* / *Novirhabdovirus*	Flatfish, cod and others	[16] [17] [18] [19]
Hirame rhabdovirus (HIRRV)	*Rhabdoviridae* / *Novirhabdovirus*	Japanese flounder	[12] [13]

Modified from Sano et al. (2011) [28]

Table 2.1. Major viruses of non-salmonid marine fish species

Lymphocystis disease virus (LCDV) belonging to the genus *Lymphocystis* (*Iridoviridae*) causes benign, wart-like lesions comprising of grossly hypertrophoid cells in the body surface of fish. The disease (lymphocystis) has been recorded in a variety of cultured/wild marine and freshwater fish species worldwide (Wolf et al, 1966). Hirame rhabdovirus (HIRRV) infection has caused mortalities in hatchery-reared juvenile Japanese flounder in Japan (Kimura et al, 1986; Nishizawa et al, 1991). The disease can be controlled experimentally by rearing fish at the water temperatures higher than 16°C (Oseko et al, 1988). Compared with these viral diseases, which have caused somewhat limited damages in some regions, the other three diseases, i.e. red sea bream iridoviral disease (RSIVD) caused by megalocytiviruses (*Iridoviridae*), viral nervous necrosis (VNN) caused by betanodaviruses (*Nodaviridae*), and viral hemorrhagic septicaemia (VHS) caused by viral hemorrhagic septicaemia virus (VHSV) belonging to the genus *Novirhabdovirus* in the family *Rhabdoviridae*, have higher geographical distribution, host range and virulence, thus, they have higher socio-economic importance in world marine aquaculture. Both RSID and VHS are listed in the aquatic animal health code by the World Organization for Animal Health (OIE, 2011). VHS has long been known as the most serious disease in farmed rainbow trout (*Oncorhynchus mykiss*) in Europe since 1950s, and the disease to date is recognized as a disease of both farmed salmonid and non-salmonid fish including a wide range of wild fish, with a global distribution (Smail and Snow 2011). Although VHS outbreaks have been reported in farmed non-salmonid marine fish, they are limited to turbot (*Scophthalmus maximus*) in Europe (Schlotfeldt et al, 1991; Ross et al, 1994) and Japanese flounder in Japan and Korea (Isshiki et al, 2001; Kim et al, 2009). In this chapter, RSIVD and VNN regarding short history of the disease, property of the virus, diagnostic methods and control of the disease are briefly described.

2.3. Red Sea Bream Iridoviral Disease (RSIVD)

Red sea bream iridoviral disease (RSIVD) was first described in farmed juvenile red sea bream (*Pagrus major*) in 1990 in Japan (Inouye et al, 1992). Currently, more than 30 cultured marine fish species have been recorded as susceptible hosts in south-western parts of Japan, mostly fish belonging to Perciformes (Matsuoka et al, 1996; Kawakami and Nakajima, 2002). OIE (2012) listed 41 host fish species including 2 hybrid species for RSIVD (OIE, 2012). Similar diseases were reported in East and Southeast Asian countries, which were caused by infectious spleen and kidney necrosis virus (ISKNV) isolated from cultured mandarin fish (*Siniperca chuatsi*) (He et al, 2000, 2001) or other related viruses (Shi et al, 2004), resulting in severe mortalities from juvenile to marketable fish. Some review papers on RSIVD have been published (Nakajima et al, 2001; Sano et al, 2011). The RSIV and ISKNV are currently classified into the genus *Megalocytivirus* of the family *Iridoviridae*, in which the *Ranavirus* and *Lymphocystivirus* genera are also included as fish-pathogenic iridoviruses. All of the present megalocytiviruses are regarded as strains of the same or closely-related viral species (Jancovich et al, 2011). Virions of the Megalocytivirus (type species: ISKNV) possess icosahedral symmetry with about 140-200 nm in diameter, and contain a single, linear dsDNA molecule; the genome size of ISKNV is 111,362 bp with 54.8% G+C content. The complete genome sequences of other megalocytiviruses have been reported, the genome sizes being approximately 112-121 kbp (Kurita et al, 2002; He et al, 2001 ; Do et al, 2004 ; Lu et al, 2005; Ao and Chen, 2006). Figure 2.1 shows RSIV virions in the spleen of the infected red sea bream (Inouye et al, 1992). RSIV are unenveloped but sensitive to ether and chloroform, suggesting the presence of a thin lipid layer. Several fish cell lines, i.e. BF-2, CHSE-214, FHM, GF, JSKG, KRE-3, RYG-2 and YTF, are available to propagate RSIV at optimal incubation temperatures of 20-25°C. Among these cell lines, GF (grunt fin) is the most permissive to the virus, but virus productivity remains at low level (105 TCID50/ml) (Nakajima and Sorimachi, 1994). Recently, a stably susceptible cell line (MFF-1) to ISKNV was reported (Dong et al, 2008).

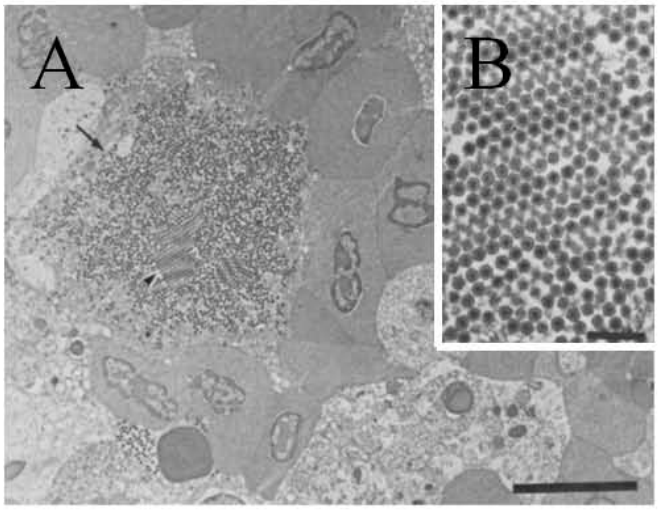

Figure 2.1. Red sea bream iridovirus (RSIV) virions in the spleen of the infected red sea bream (Inoue et al. 1992).

RSIVD occurs in the summer season at water temperature of 25°C and above in Japan (Matsuoka et al, 1996; Kawakami and Nakajima, 2002; Jun et al, 2009). Juveniles are more susceptible than adults. However, the disease has never been observed in hatcheries (Muroga, 2001). Gloss findings of the affected red sea bream are severe anemia, petechiae of the gills and enlargement of the spleen. The disease is most characterized by the presence of enlarged cells in the spleen, heart, kidney, intestine and gills of the affected fish. The enlarged cells (about 20 μm in diameter) are basophilic and Feulgen-positive, and may originate in infected leucocyte (Inouye et al, 1992). Cell culture isolation of the virus is not reliable due to lower permissibility of the cultured cells. IFAT with a specific monoclonal antibody (named M10) is a simple and reliable diagnostic method in overt infection cases of RSIV (Nakajima and Sorimachi, 1995; Nakajima et al, 1995). OIE manual (2012) recommends spleen as the most appropriate organ in the IFAT. Many PCR and nested PCR methods have been reported to identify and detect megalocytiviruses (Kurita et al, 1998; He et al, 2001; Chao et al, 2002; Jeong et al, 2003). A real-time PCR assay and LAMP method have been developed for RSIV detection (Caipang et al, 2003, 2004). Water borne route is the principal mode of virus transmission. Virus-harboring trash fish may be hazardous to farmed susceptible fish (Kim et al, 2007). RSIV is inactivated by a treatment at 55°C for 30 min, and ISKNV is susceptible to some disinfectants (Nakajima and Sorimachi, 1994; He et al, 2002). The effectiveness of formalin-killed vaccine by intraperitoneal injection was demonstrated in red sea bream under laboratory and field settings (Nakajima et al, 1997, 1999). The inactivated vaccine by injection method has been licensed in Japan since 1999, and to date is available for red sea bream, striped jack (*Pseudocaranx dentex*), Malabar grouper (*E. malabaricus*), orange-spotted grouper (*E. coioides*) and fish species of the genus Seriola. However, the vaccine is not effective to protect fish of the genus *Oplegnathus* from RSIVD. RSIVD is of great importance for aquacultures particularly in East and Southeast Asian countries, and there still remain many subjects to be studied in the future.

2.4. Viral Nervous Necrosis (VNN)

Viral nervous necrosis (VNN), also known as viral encephalopathy and retinopathy (VER), is one of the most important limiting factors in successful seed production in marine aquaculture worldwide, because of its wide range of host species and severe mortalities, often up to 100%. The disease was first described in 1990 in hatchery-reared Japanese parrotfish (*Oplegnathus fasciatus*) in Japan and Asian sea bass (barramundi, *Lates calcarifer*) in Australia (Yoshikoshi and Inoue, 1990; Glazebrook et al, 1990). In the following 1991-1992 years, the disease was documented in larvae of turbot (*Scophthalmus maximus*) in Norway, European sea bass (*Dicentrarchus labrax*) in France and striped jack (*Pseudocarnx dentex*) in Japan, and in larvae/juveniles of red-spotted grouper (*Epinephelus akaara*) in Japan (Bloch et al, 1991; Breuil et al, 1991 ; Mori et al, 1991 ; Mori et al, 1992).

Although the disease is most serious during hatchery-rearing period, several fish species such as sevenband grouper, European sea bass, Atlantic halibut (*Hippoglossus hippoglossus*) and Atlantic cod (*Gadus morhua*) remain susceptible to the causative virus through older stages (Fukuda et al, 1996; Le Breton et al, 1997 ; Aspehaug et al, 1999 ; Patel et al, 2007). The number of host fish species of VNN is still increasing, to date reached more than 40 species (24 families and 8 orders). Some instances of the disease in

freshwater fish species or freshwater farms have been recorded. No overt VNN has been observed in salmonids, though Atlantic salmon (*Salmo salar*) is susceptible to experimental infection (Korsnes et al, 2005). Some review papers have been published for VNN and the causative agents (Munday et al, 2002; Sano et al, 2011). The causative agent of VNN was first characterized in diseased larval striped jack (*Pseudocaranx dentex*), named as striped jack nervous necrosis virus (SJNNV), and identified as a member of the family *Nodaviridae* (Mori et al, 1992). Agents in diseased larval Asian sea bass and European sea bass were also purified and identified as nodavirus (Comps et al, 1994; Chi et al, 2001). The current virus taxonomy classifies piscine nodaviruses into the genus *Betanodavirus*, while the genus *Alphanodavirus* is for insect nodaviruses (Thiéry et al, 2012). Betanodaviruses are non-enveloped and round-shaped with 25-30 nm in diameter. The genome consists of two positive sense ssRNA molecules; RNA1 (RNA-dependent RNA polymerase gene: 3.1 kb) and RNA2 (coat protein gene: 1.4 kb). Both molecules lack poly (A) tails at their 3'-ends. A subgenomic RNA3 (0.4 kb), which is derived from RNA1 in infected cells, encodes a protein with an RNA silencing-suppression activity. Based on the coat protein gene sequences, betanodaviruses are subgrouped into four major genotypes; designated SJNNV (type species of the genus), TPNNV, RGNNV and BFNNV (Nishizawa et al, 1995, 1997).

Most of isolates from diseased fish are either RGNNV genotype or BFNNV genotype. Complete nucleotide genome sequences were reported for SJNNV, RGNNV and BFNNV (Iwamoto et al, 2001; Tan et al, 2001; Sommerset and Nerland, 2004;), and the established infectious RNA transcription system demonstrated that viral RNA2 and/or encoded coat protein control host specificity in SJNNV and RGNNV (Iwamoto et al, 2004). These genotypes are related closely to the serotypes, i.e. serotype A for SJNNV, serotype B for TPNNV and serotype C for both BFNNV and RGNNV (Mori et al, 2003). There are also a close relationship between the genotypes and the host fish from which virus was isolated. The RGNNV has been isolated from warm water fish, while the BFNNV has been isolated from cold water fish, suggesting that water temperature is an important factor influencing disease outbreaks (Iwamoto et al, 1999). Figure 2.2 shows the purified RGNNV virions which were isolated from the diseased sevenband grouper. There are no clinical signs on the body surface and gills of affected fish at any stages. The affected juveniles or older fishes are characterized by a range of erratic swimming behaviors: spiral, whirling movement or belly-up floating with inflation of swim bladder, or lying down at rest. Histopathologically, severely extended necrosis and vacuolation are observed in the affected brain, spinal cord and retina (Figure 2.3). PCR-based methods are most rapid and convenient to diagnose clinically affected fish. For a confirmative diagnosis, however, either histopathology or virus isolation in cell culture followed by immunological procedures (FAT, IHC, ELISA) or sequencing analysis has to be performed. SSN-1 and E-11 cell lines are now officially provided to isolate and propagate any of betanodaviruses (Frerichs et al, 1996; Iwamoto et al, 2000). PCR-based or cell culture isolation method demonstrated that betanodaviruses are frequently detected in asymptomatic cultured and wild marine fish including trash fish (Castric et al, 2001; Barker et al, 2002; Gomez et al, 2004). These subclinically or persistently infected fish might be important as a potential infection source for farmed fish susceptible to the virus.

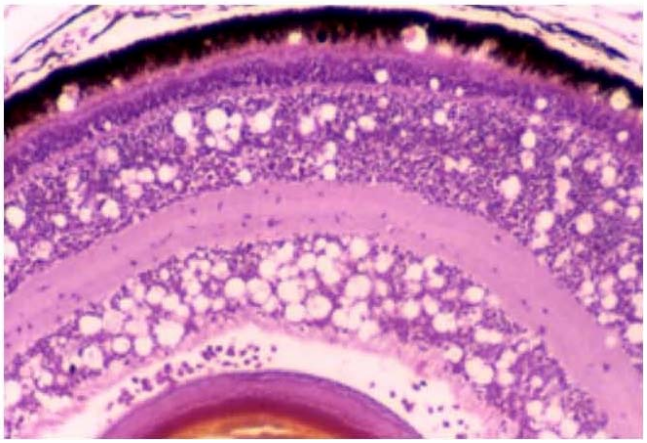

Figure 2.2. Purified betanodavirus (RGNNV) virions from the diseased sevenband grouper. Photograph by K. Mori (NRIA, Japan).

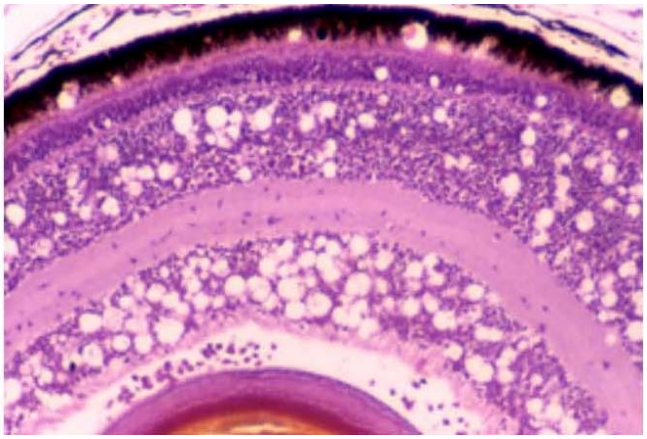

Figure 2.3. Extensive vacuolation characteristic to VNN in the retina of the diseased red-spotted grouper

Disinfectants (iodine, ozone and sodium hypochlorite) are useful to prevent water-borne transmission of the virus (Arimoto et al, 1996; Frerichs et al, 2000). For prevention of vertical transmission from broodstock to off-spring, elimination of virus-carrying broodstocks by the PCR-based methods and disinfection of fertilized eggs and rearing water by disinfectants are successful (Mushiake et al, 1994; Mori et al, 1998 ; Grotmol et al, 2000). On the other hand, establishment of the effective vaccination system has been studied for a long time to reduce severe economic damage during net-pen culture in the open sea. Different types of vaccine candidate by injection have been reported to be effective against VNN, e.g. recombinant capsid protein, inactivated virion and virus-like particles (Húsgard et al, 2001 ; Tanaka et al, 2001; Thiéry et al, 2006 ; Yamashita et al, 2005 ; Yamashita et al, 2009). The efficacy of a bath immunization with inactivated vaccine was also reported in larval orange-spotted grouper (*E. coioides*) (Kai and Chi, 2008). In 2012, an inactivated injection vaccine against RGNNV infection of sevenband grouper (*E. septemfasciatus*) was commercialized in Japan. This vaccine is possibly effective to VNN in other fish species due to not only RGNNV but also BFNNV because of their serological similarity (Mori et al, 2003).