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Current Knowledge on Viral Nervous Necrosis (VNN) and its Causative Betanodaviruses

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Abstract

Viral nervous necrosis (VNN) or viral encephalopathy and retinopathy (VER) caused by betanodaviruses (*Nodaviridae*) has seriously damaged global marine aquaculture since its first appearance in the late 1980s. In the past two decades, more than 100 papers have been published on the disease. Although information is still limited, we now have more knowledge on the taxonomic position and molecular characteristics of betanodaviruses, and on the diagnosis, control, and infection mechanisms of the disease. This paper briefly reviews studies on VNN and betanodaviruses.

Introduction

Viral nervous necrosis (VNN; Yoshikoshi and Inoue, 1990), also known as viral encephalopathy and retinopathy (VER; OIE, 2003), is caused by piscine nodaviruses. It was first described in 1990 in hatchery-reared Japanese parrotfish *Oplegnathus fasciatus* in Japan (Yoshikoshi and Inoue, 1990) and barramundi (Asian sea bass) *Lates calcarifer* in Australia (Glazebrook et al., 1990). Later, it was reported in turbot *Scophthalmus maximus* (Bloch et al., 1991), European sea bass *Dicentrarchus labrax* (Breuil et al., 1991), redspotted grouper *Epinephelus akaara* (Mori et al., 1991), and striped jack *Pseudocaranx dentex* (Mori et al., 1992). Thereafter, the disease was documented in a variety of cultured warm-water and cold-water marine fish species throughout the world (Munday et al., 2002). Bibliographically, the disease may be traced to a case of mass mortality of European sea bass larvae and juveniles in French Martinique (Bellance and Gallet de Saint-Aurin, 1988).

The causative agent of VNN was first purified from diseased striped jack larvae, then characterized and identified as a new member of the *Nodaviridae* family with the name striped jack nervous necrosis virus (SJNNV; Mori et al., 1992). Another agent with the same characteristics as SJNNV

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was purified from diseased Asian and European sea bass larvae (Comps et al., 1994) and diseased larvae of a grouper of the *Epinephelus* sp. (Chi et al., 2001). The nodaviruses isolated from fish are phylogenetically different from nodaviruses isolated from insects (Nishizawa et al., 1995), and the fish viruses were classified as the genus *Betanodavirus* within the family *Nodaviridae* in the 7th report of the International Committee on Taxonomy of Viruses (ICTV; Ball et al., 2000).

Almost two decades have passed since the first appearance of VNN in aquaculture. One of the most epoch-making events in VNN and betanodavirus research was the establishment of a cell line (SSN-1) to propagate betanodaviruses (Frerichs et al., 1996). Then a number of fish cell lines were established for the virus. In addition to propagation of the virus, these lines made it possible to quantitatively analyze infectivity, which accelerated all research activities on VNN and betanodaviruses. Review papers on betanodavirus infections in fish were published (Munday and Nakai, 1997; Muroga et al., 1998; Munday et al., 2002) and the first international symposium on viral nervous necrosis of fish was held in Japan in 2006.

We have gained much knowledge on the taxonomic position of betanodaviruses and their phenotypic and genotypic characteristics, infectivity and host specificity of the virus, infection and transmission mechanisms of the disease, and diagnostic and control measures. But insufficient information still limits our full understanding. This paper reviews previous studies on VNN and betanodaviruses.

Geographic Distribution

VNN has been reported on all continents except South America (Johnson et al., 2002; Munday et al., 2002; Azad et al., 2005). These include south and east Asia (Japan, Korea, Taiwan, China, Philippines, Thailand, Vietnam, Malaysia, Singapore, Indonesia, Brunei, India), Oceania (Australia, Tahiti), the Mediterranean (Israel, Croatia, Bosnia, Greece, Malta, Italy, France, Spain, Portugal, Tunisia), the UK, Scandinavia (Norway), and North America (USA, Canada).

Host Fish Species

The number of fish species affected by VNN is steadily increasing. In an early brief review of the disease, 19 fish species (10 families, 3 orders), including Japanese parrotfish, redspotted grouper, striped jack, barramundi, turbot, and European sea bass, were described as hosts of VNN (Munday and Nakai, 1997). In the next full-dress review paper, 32 species (16 families, 5 orders) were listed (Munday et al., 2002). In more recent publications and unpublished data, 39 host species are included (22 families, 8 orders). Newly added host fish include sturgeon *Acipenser* sp., Chinese catfish *Parasilurus asotus*, guppy *Poecilia reticulata*, dragon grouper *Epinephelus lanceolatus*, Japanese tilefish *Branchiostegus japonicus*, firespot snapper *Lutjanus erythropterus*, and bluefin tuna *Thunnus thynnus*.

Most fish are affected at larvae or juvenile stages, but significant mortality occurs in older fish up to production-size in European sea bass, sevenband grouper, and Atlantic halibut *Hippoglossus hippoglossus* (Fukuda et al., 1996; Le Breton et al., 1997; Aspehaug et al., 1999). Instances of natural infection in freshwater fish species or freshwater farms have been documented (Chi et al., 1999; Hegde et al., 2003; Athanassopoulou et al., 2004). Experimental infections show that other saltwater and freshwater fishes are susceptible to the virus (Johansen et al., 2003; Furusawa et al., 2006, 2007), suggesting potential new hosts.

Clinical Signs and Features

The disease occurs mostly at early developmental stages of fish (larvae and juveniles). There are no gross clinical signs on the body surface or gills at any stage. Affected juveniles and older fish show a variety of erratic swimming behaviors such as spiral, whirling, belly-up floating with inflation of swim bladder, or laying down at rest, but it is unusual to find such swimming abnor-

malities in affected larvae. Histopathologically, the disease is characterized by severely extended necrosis and vacuolation of the central nervous system (brain, spinal cord) and retina, but sometimes fish in early larval stages lack tissue vacuolation (Munday et al., 2002).

There are considerable variations of age at which the disease is first noted and period during which mortality occurs (Munday et al., 2002; OIE, 2003). In general, the earlier the signs of disease occur, the greater is the rate of mortality. In striped jack, the earliest occurrence is one day post-hatching and the latest occurrence of new outbreaks is 20 days post-hatching (8 mm total length), resulting in almost complete loss of larvae (Mori et al., 1992; Arimoto et al., 1994). In European sea bass, mortality is usually not seen until about 30 days post-hatching and new disease outbreaks occur even in market-size fish (Le Breton et al., 1997). Affected market-size sevenband grouper *Epinephelus septemfasciatus* (up to 1.8 kg body weight) exhibit belly-up swimming and inflation of the swim bladder (Fukuda et al., 1996). Mortality of juvenile or older fish usually does not reach 100%, indicating the age-dependence of susceptibility.

Causative Virus

Betanodaviruses are non-enveloped and spherical (ca. 25-30 nm diameter). The genome consists of two molecules of positive sense ssRNA: RNA1 (3.1 kb) encodes the replicase (110 kDa) and RNA2 (1.4 kb) encodes the coat protein (42 kDa). Both molecules lack poly (A) tails at their 3'-ends (Mori et al., 1992; Comps et al., 1994; Chi et al., 2001; Schneemann et al., 2005). A subgenomic RNA3 (0.4 kb) is derived from RNA1 in infected cells (Somerset and Nerland, 2004) and encodes a protein with a potent RNA silencing-suppression activity (Iwamoto et al., 2005). Complete nucleotide sequences of RNA1 and RNA2 were reported for betanodaviruses isolated from striped jack (Iwamoto et al., 2001), greasy grouper *Epinephelus tauvina* (Tan et al., 2001), and sevenband grouper (Iwamoto et al., 2004; Fig. 1).

Based on the similarity of partial RNA2 sequences (ca. 380 bases), betanodaviruses are classified into four major genotypes (Nishizawa et al., 1995): designated striped jack nervous necrosis virus (SJNNV), tiger puffer nervous necrosis virus (TPNNV), barfin flounder nervous necrosis virus (BFNNV), and redspotted grouper nervous necrosis virus (RGNNV). The genetic differences are closely related to the serotypes by neutralization with polyclonal antibodies, i.e., serotype A for SJNNV-type, serotype B for TPNNV-type, and serotype C for both BFNNV- and RGNNV-types (Mori et al., 2003; Table 1).

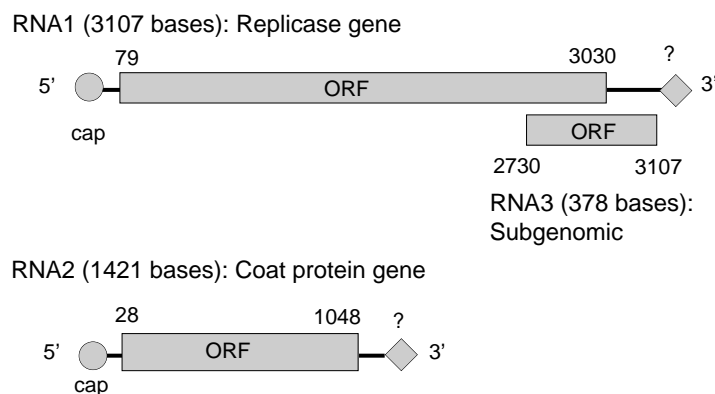


Fig. 1. Genome organization of striped jack nervous necrosis virus (SJNNV, type species of the Betanodavirus; Iwamoto et al., 2001).

Table 1. Genotypic and phenotypic variations of betanodaviruses

<i>Genotype</i> ¹	<i>Serotype</i> ²	<i>Major fish hosts</i>	<i>Optimum in vitro growth temperature</i> ³
Striped jack nervous necrosis virus (SJNNV)	A	Striped jack	20-25°C
Tiger puffer nervous necrosis virus (TPNNV)	B	Tiger puffer	20°C
Redspotted grouper nervous necrosis virus (RGNNV)	C	Asian sea bass European sea bass Redspotted grouper Other groupers	25-30°C
Barfin flounder nervous necrosis virus (BFNNV)	C	Atlantic cod Atlantic halibut Barfin flounder Japanese flounder	15-20°C

¹ Nishizawa et al. (1995)² Mori et al. (2003)³ Iwamoto et al. (2000)

Host fish are also closely related to genotype (Table 1). RGNNV has been isolated from diseased warm-water fishes, while BFNNV has been isolated from diseased cold-water fishes (Nishizawa et al., 1997; Aspehaug et al., 1999; Iwamoto et al., 1999; Thiery et al., 1999; Skliris et al., 2001; Johnson et al., 2002; Chi et al., 2003), suggesting that water temperature is an important factor influencing disease outbreaks. This is partly supported by *in vitro* virus proliferation studies showing the optimum growth temperature of the virus in cultured cells (Iwamoto et al., 2000; Lai et al., 2001; Ciulli et al., 2006; Hata et al., 2007). No difference was noticed in mortality caused by SJNNV infection in striped jack larvae reared in water of 20-26°C, while water temperature of 16-28°C influenced development of RGNNV disease in redspotted grouper. Higher mortality and earlier appearance of the disease in sevenband grouper were observed at higher temperatures (Tanaka et al., 1998), but water temperature above 31°C inhibited the proliferation of RGNNV in humpback grouper *Cromileptes altivelis* (Yuasa et al., 2007). On the other hand, infection of Atlantic halibut by Atlantic halibut nodavirus (BFNNV genotype) occurred at 6°C (Grotmol et al., 1999).

Diagnosis

Presumptive diagnosis of VNN is based on the appearance of vacuoles in the brain, spinal cord, and/or retina as seen by light microscopy. However, individual fish showing only a few vacuoles in the nervous tissues pose a difficulty in diagnosis. In electron microscopy, the virus is mainly associated with vacuolated cells and arranged intracytoplasmically in paracrystalline arrays or as aggregates. Confirmatory diagnosis is made by immuno-staining methods, fluorescent antibody test (FAT), or immunohistochemistry (IHC), using polyclonal or monoclonal antibodies (Nguyen et al., 1996; Grotmol et al., 1999; OIE, 2003).

Reverse-transcriptase polymerase chain reaction (RT-PCR) is the most rapid and convenient method of diagnosing clinically affected fish. Nested PCR is particularly useful to diagnose

asymptomatic fish (broodstock). There are a number of RT-PCR and nested PCR methods with different primers and protocols, but the methods are not standardized (Nishizawa et al., 1994; Thiery et al., 1999; Grotmol et al., 2000; Huang et al., 2001; Gomez et al., 2004; Cutrin et al., 2007). Among them, the primer set R3 and F2 (Nishizawa et al., 1994) is designed to amplify the variable region (ca. 380 bases) of the SJNNV coat protein gene and is useful for detecting almost all genotypic variants, with only one exception (Thiery et al., 1999). However, PCRs must be followed by another method, either histopathology with immuno-staining or virus isolation in cell culture, for confirmative diagnosis.

Several fish cell lines are now available to isolate and propagate betanodaviruses: SSN-1 (Frerichs et al., 1996) and E-11 (Iwamoto et al., 2000) derived from striped snakehead *Ophicephalus striatus*, GF-1 (Chi et al., 1999) and other cell lines from groupers (Lai et al., 2001, 2003), and TV-1 and TF from turbot (Aranguren et al., 2002). Virus identification in cell culture showing cytopathic effects (CPE) is performed by either molecular or serological assay including a virus neutralizing test (Mori et al., 2003).

Control

VNN is highly resistant to various environmental conditions and can survive for a long time in sea water (Arimoto et al., 1996; Frerichs et al., 2000). The disease is easily reproduced in healthy fish by co-habitation with infected fish, immersion in a virus suspension, or injection (Nguyen et al., 1996; Grotmol et al., 1999; Peducasse et al., 1999). Thus, the mode of transmission of infection is basically horizontal through influent and rearing water, and via utensils, vehicles, and human activity. To prevent such horizontal transmission, some disinfectants are effective in inactivating the virus: sodium hypochlorite, benzalkonium chloride, iodine, acid peroxygen, and ozone (Arimoto et al., 1996; Frerichs et al., 2000).

There is evidence of vertical transmission of infection from broodstock to offspring in striped jack, European sea bass, Asian sea bass, barfin flounder, Atlantic halibut, and sevenband grouper (Arimoto et al., 1992; Comps et al., 1994; Mushiake et al., 1994; Watanabe et al., 2000). Washing fertilized eggs in ozone-treated sea water and disinfection of rearing water by ozone effectively control the disease in larvae production of striped jack, barfin flounder, Atlantic halibut, and sevenband grouper (Mori et al., 1998; Watanabe et al., 1998; Grotmol and Totland, 2000; Tsuchihashi et al., 2002). It is important to reduce stress factors by improving spawning induction methods including broodstock feed (Mushiake et al., 1994). The Japan Sea-Farming Association (JASFA) began to investigate VNN in striped jack larvae in 1990 and succeeded to control the disease by eliminating virus-carrying broodstocks and disinfecting fertilized eggs and rearing water (Mori et al., 1998; Mushiake and Arimoto, 2000; Fig. 2). The established procedures proved effective for VNN in sevenband grouper larvae (Tsuchihashi et al., 2002).

Some fish species, such as groupers and sea bass, are highly susceptible to RGNNV in grow-out stages and VNN causes severe mortality in net-pen culture in the open sea. Infection may be caused by virus shedding from subclinically or persistently infected cultured or wild fish (Castric et al., 2001; Barker et al., 2002; Johansen et al., 2003; Gomez et al., 2004; Chi et al., 2005; Cutrin et al., 2007; Sakamoto et al., 2008). An efficacious vaccination system is essential to prevent the disease during grow-out stages in net-pen culture. Injection with a recombinant viral coat protein expressed in *Escherichia coli* (Husgard et al., 2001; Tanaka et al., 2001), or virus-like particles expressed in a baculovirus expression system (Thiery et al., 2006), or inactivated virus (Yamashita et al., 2005) is effective in controlling the disease. A primary infection with an avirulent aquabirnavirus (*Birnaviridae*) effectively suppressed a secondary betanodavirus infection (Pakingking et al., 2005). Double inoculations with aquabirnavirus and inactivated vaccine are very useful in preventing the disease (Yamashita et al., 2009). Commercially available vaccine preparations are urgently needed.

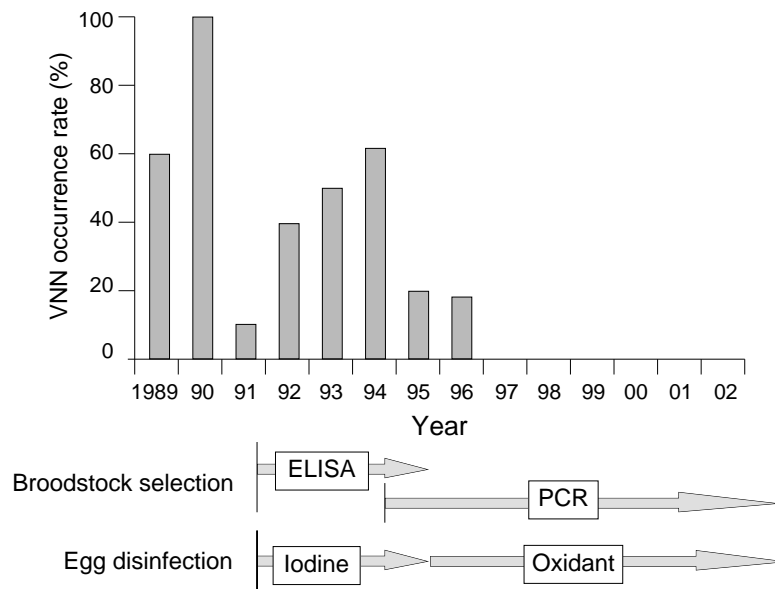


Fig. 2 Control of viral nervous necrosis (VNN) in production of striped jack larvae by the Japan Sea-Farming Association (JASFA; Mori et al., 1998, modified).

Future Directions

More than 100 papers on VNN infections and related subjects have been published in the past two decades. Accumulated knowledge on the disease and causative betanodaviruses have led to the development of useful diagnostic and control methods. However, much remains to be done to reveal the molecular characteristics of the virus, interactions between host fish and the virus, and transmission mechanisms in natural environments. Practical control procedures for the disease, especially to reduce severe economic damage during net-pen culture in the open sea, remain to be established.

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